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FILE 'MEDLINE' ENTERED AT 16:37:32 ON 09 OCT 2002

=> s ammonium
L1      631272 AMMONIUM

=> s catabolic
L2       31662 CATABOLIC

=> s l1 and l2
L3        1022 L1 AND L2

=> s muscle or protein
   4 FILES SEARCHED...
L4     7111621 MUSCLE OR PROTEIN

=> s l3 and l4
L5         704 L3 AND L4

=> s l5 and py<2001
   4 FILES SEARCHED...
L6         474 L5 AND PY<2001

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7          433 DUP REM L6 (41 DUPLICATES REMOVED)

=> s ammonium salt or ammonium chloride
L8      176334 AMMONIUM SALT OR AMMONIUM CHLORIDE

=> s l7 and l8
L9         144 L7 AND L8

=> d l9 1-10 ab bib kwic

L9   ANSWER 1 OF 144  CAPLUS  COPYRIGHT 2002 ACS
AB   The effect of acidosis on whole body protein turnover was detd.
     from the kinetics of infused L-[1-13C]leucine.  Seven healthy subjects
     were studied before (basal) and after (acid) the induction of acidosis
     with 5 days oral ammonium chloride (basal pH 7.42,
     acid pH 7.35).  Bicarbonate recovery, measured from the kinetics of
     infused NaH13CO3, was increased in the acidotic state (basal 72.9 vs.
acid
     77.6%).  Leucine appearance from body protein (PD), leucine
     disappearance into body protein (PS), and leucine oxidn. (O)
     increased.  Plasma levels of the amino acids threonine, serine,
     asparagine, citrulline, valine, leucine, ornithine, lysine, histidine,
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arginine, and hydroxyproline increased with the induction of acidosis. Thus, acidosis in humans is a **catabolic** factor stimulating **protein** degrdn. and amino acid oxidn.

AN 1993:122336 CAPLUS
DN 118:122336
TI **Ammonium chloride**-induced acidosis increases **protein** breakdown and amino acid oxidation in humans
AU Reaich, David; Channon, Susan M.; Scrimgeour, Charles M.; Goodship, Timothy H. J.
CS Dep. Med., Univ. Newcastle upon Tyne, Newcastle upon Tyne, NE1 4LP, UK
SO American Journal of Physiology (**1992**), 263(4, Pt. 1), E735-E739
CODEN: AJPHAP; ISSN: 0002-9513
DT Journal
LA English
TI **Ammonium chloride**-induced acidosis increases **protein** breakdown and amino acid oxidation in humans
SO American Journal of Physiology (**1992**), 263(4, Pt. 1), E735-E739
CODEN: AJPHAP; ISSN: 0002-9513
AB The effect of acidosis on whole body **protein** turnover was detd. from the kinetics of infused L-[1-13C]leucine. Seven healthy subjects were studied before (basal) and after (acid) the induction of acidosis with 5 days oral **ammonium chloride** (basal pH 7.42, acid pH 7.35). Bicarbonate recovery, measured from the kinetics of infused NaH13CO3, was increased in the acidotic state (basal 72.9 vs. acid 77.6%). Leucine appearance from body **protein** (PD), leucine disappearance into body **protein** (PS), and leucine oxidn. (O) increased. Plasma levels of the amino acids threonine, serine, asparagine, citrulline, valine, leucine, ornithine, lysine, histidine, arginine, and hydroxyproline increased with the induction of acidosis. Thus, acidosis in humans is a **catabolic** factor stimulating **protein** degrdn. and amino acid oxidn.
ST **protein** degrdn amino acid oxidn acidosis
IT Acidosis
(amino acid oxidn. and **protein** breakdown in, in human)
IT Amino acids, biological studies
Proteins, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(metab. of, in human acidosis)

L9 ANSWER 2 OF 144 USPATFULL
AB A selection method for selecting from a population of plant cells one or more genetically transformed plant cells is described. In the method, the population of plant cells includes selectable genetically transformed plant cells and possible non-transformed plant cells. Each of the selectable genetically transformed plant cells comprises a first expressible nucleotide sequence and optionally a second expressible nucleotide sequence. In the method, a component or a metabolic derivative thereof when present in a low concentration in a medium is a nutrient for both the selectable genetically transformed plant cells and the non-transformed plant cells. In the method, the component or the metabolic derivative thereof when present in a high concentration in a medium is toxic to the non-transformed plant cells. The first nucleotide sequence codes for a gene product having glucosamine-6-phosphate deaminase activity which is capable of converting the component or the metabolic derivative thereof when present in a high concentration in a

medium to a nutrient for the selectable genetically transformed plant cells. The method includes the step of introducing the population of plant cells to a medium, wherein the medium includes a high concentration of the component or the metabolic derivative thereof. In the method, the component or the metabolic derivative thereof is a source of both carbohydrate and nitrogen for the selectable genetically transformed plant cells.

AN 2002:224767 USPATFULL
TI Method of plant selection using glucosamine-6-phosphate deaminase
IN Donaldson, Iain A., Tinglev, DENMARK
Bojsen, Kirsten, Allerod, DENMARK
Jorgensen, Kirsten, Guldborg, DENMARK
Jorsboe, Morten, Nykobing Falster, DENMARK
PA Danisco A/S, Copenhagen, DENMARK (non-U.S. corporation)
PI US 6444878 B1 20020903
WO 9835047 19980813 <--
AI US 1999-367293 19991223 (9)
WO 1998-GB367 19980205
19991223 PCT 371 date
PRAI GB 1997-2592 19970207
DT Utility
FS GRANTED
EXNAM Primary Examiner: Fox, David T.; Assistant Examiner: Kruse, David H
LREP Knobbe, Martens, Olson & Bear, LLP
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 28 Drawing Figure(s); 28 Drawing Page(s)
LN.CNT 2614
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6444878 B1 20020903
WO 9835047 19980813 <--
SUMM . . . natural to the cell or organism (e.g. a particular plant) in question. Typical examples of a NOI include genes encoding **proteins** and enzymes that modify metabolic and **catabolic** processes. The NOI may code for an agent for introducing or increasing resistance to pathogens. The NOI may even be. . . hemicellulases, endo-.beta.-glucanases, arabinases, or acetyl esterases, or combinations thereof, as well as antisense sequences thereof. The NOI may encode a **protein** giving nutritional value to a food or crop. Typical examples include plant **proteins** that can inhibit the formation of anti-nutritive factors and plant **proteins** that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant).

SUMM . . . the first nucleotide sequence. This additional, selection nucleotide sequence may be an additional gene coding for an enzyme (or other **protein** or polypeptide) suitable for selection according to the present invention, or it may be a gene coding for an enzyme (or other **protein** or polypeptide) for a known selection method, eg coding for resistance to a antibiotic or herbicide or it may be. . .

DETD . . . nitrogen source for the transformed cells. The glucosamine can even used as a supplement for decreased levels of sucrose and **ammonium salts** in the tissue culture medium. At present, it is believed that the selection system has the added benefit of being. . .

L9 ANSWER 3 OF 144 USPATFULL
AB A method for synthesizing C-glycosides of ulosonic acids such as Neu5Ac,

by which diastereocontrolled synthesis of .alpha.-C-glycosides of ulosonic acids is attained is disclosed. In the method of the present invention, an ulosonic acid sulfone or phosphite is reacted with an aldehyde or ketone compound in the presence of a lanthanide metal halide.

AN 2002:88629 USPATFULL
TI Method for synthesizing C-glycosides of ulosonic acids
IN Linhardt, Robert J., Iowa City, IA, United States
Vlahov, Iontcho R., Newark, DE, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 6376662 B1 20020423
WO 9831696 19980723 <--
AI US 1998-142937 19981117 (9)
WO 1998-JP129 19980116
19981117 PCT 371 date
PRAI US 1997-34953P 19970117 (60)
US 1997-35969P 19970121 (60)
US 1997-35986P 19970123 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Woodward, Michael P; Assistant Examiner: Moran, Marjorie A.
LREP Birch, Stewart, Kolasch & Birch, LLP
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 649
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6376662 B1 20020423
WO 9831696 19980723 <--
SUMM . . . . oligosaccharides and suppressing undesired immune reactions (antirecognition phenomena); influencing the cell membrane permeability for permeability for ions, amino acids and **proteins**; and protection of glycoproteins against proteolysis.sup.1. Terminal Neu5Ac is an attachment site of pathogens to the cells and often **catabolic** and inflammatory processes are initiated on the removal of this carbohydrate group.sup.2. In general the "right" life time of a. . .
DETD . . . . (Merck); detection under short wavelength UV light (254 nm) and
by dipping the plates into staining solution (1.0 g ceric **ammonium** sulfate and 24.0 g **ammonium** molybdate in 31 mL sulfuric acid 470 mL water) then heating. Flash chromatography was performed using 230-400 mesh silica gel. . . .
DETD . . . . added dropwise at 20.degree. C. Stirring was continued for 45 min, than the reaction mixture was poured into an aqueous **ammonium chloride** solution and extracted twice with ethyl acetate. The combined organic layers were dried (MgSO.sub.4) and concentrated in vacuo. The residue. . .
DETD . . . . added dropwise at 20.degree. C. Stirring was continued for 45 min, than the reaction mixture was poured into an aqueous **ammonium chloride** solution and extracted twice with ethyl acetate. The combined organic layers were dried (MgSO.sub.4) and concentrated in vacuo. The residue. . .
DETD . . . . added dropwise at 20.degree. C. Stirring was continued for 45 min, than the reaction mixture was poured into an aqueous **ammonium chloride** solution and extracted twice with ethyl acetate. The combined organic layers were dried (MgSO.sub.4) and concentrated in vacuo. The residue. . .

DETD (3) Air, G. M.; Laver, W. G. **Proteins**: Structure, Function and Genetics, 1989, 6, 341-356, and references therein.

L9 ANSWER 4 OF 144 USPATFULL
AB The present invention is directed to methods for the modulation of cardiac function which comprise the administration of certain compounds,
as defined herein, having growth hormone secretagogue activity.
AN 2001:226599 USPATFULL
TI Treatment of congestive heart failure with growth hormone secretagogues
IN Kauffman, Raymond F., Carmel, IN, United States
Palkowitz, Alan D., Carmel, IN, United States
PA Eli Lilly and Company, Indianapolis, IN, United States (U.S. corporation)
PI US 6329342 B1 20011211
WO 9908697 19990225 <--
AI US 2000-485924 20000218 (9)
WO 1998-US17201 19980819
20000218 PCT 371 date
20000218 PCT 102(e) date
PRAI US 1997-56135P 19970819 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Henley, III, Raymond
LREP Boudreaux, William R., Strode, Janelle D., McNeil, Scott A.
CLMN Number of Claims: 33
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 14373
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6329342 B1 20011211
WO 9908697 19990225 <--
SUMM . . . forms of heart disease. Common causes of congestive heart failure include: narrowing of the arteries supplying blood to the heart **muscle** (coronary heart disease); prior heart attack (myocardial infarction) resulting in scar tissue large enough to interfere with normal function of. . . pressure; heart valve disease due to past rheumatic fever or an abnormality present at birth; primary disease of the heart **muscle** itself (cardiomyopathy); defects in the heart present at birth (congenital heart disease) and infection of the heart valves and/or **muscle** itself (endocarditis and/or myocarditis). Each of these disease processes can lead to congestive heart failure by reducing the strength of the heart **muscle** contraction, by limiting the ability of the heart's pumping chambers to fill with blood due to mechanical problems or impaired. . .
SUMM . . . hormone is known to have the following basic effects on the metabolic processes of the body: (1) increased rate of **protein** synthesis in all cells of the body; (2) decreased rate of carbohydrate utilization in cells of the body; and (3). . .
SUMM . . . also known as dwarfism. A deficiency in growth hormone secretion later in life may be characterized by excessive adiposity, reduced **muscle** mass, impaired exercise capacity, reduced body water, decreased bone mineral density, and psychological disorders. For example, a deficiency in growth. . .
DETD Salts of amine groups may also comprise quaternary **ammonium salts** in which the amino nitrogen carries a suitable organic group such as an alkyl, alkenyl, alkynyl, or aralkyl moiety.
DETD Base addition salts include those derived from inorganic bases, such as **ammonium** or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing

the salts of this invention thus include sodium hydroxide, potassium hydroxide, **ammonium** hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide,
calcium carbonate, and the like. The potassium and sodium. . .

DETD . . . another composition which exhibits another activity, for example an antibiotic growth promoting agent, or a corticosteroid employed to minimize the **catabolic** side effects, or another compound which enhances efficacy and minimizes side effects. Growth promoting and anabolic agents include TRH, diethylstilbesterol,. . .

DETD . . . as agar agar, calcium carbonate, and sodium bicarbonate; agents
for retarding dissolution such as paraffin; resorption accelerators such
as quaternary **ammonium** compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such. . .

DETD . . . dichloromethane (500 mL) at 0.degree. C. was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (12.3 g, 71.9 mmol). After
18 h, ethyl acetate and saturated **ammonium chloride** were added and the mixture extracted with **ammonium chloride**, sodium bicarbonate, and brine. The organic extracts were dried over sodium sulfate and concentated. Purification by silica gel chromatography (25%. . .

DETD . . . and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (1.36 g, 7.93
mmol). After 18 hours, ethyl acetate was added and the mixture washed with saturated aqueous **ammonium chloride**, saturated aqueous sodium bicarbonate, and brine. The organic extract was dried over sodium sulfate and concentrated. Purification by silica gel. . .

DETD . . . dichloromethane (500 mL) at 0 .degree. C. was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (12.3 g, 71.9 mmol). After
18 h, ethyl acetate and **ammonium chloride** (saturated aqueous solution) were added and the resulting mixture extracted with aqueous **ammonium chloride**, aqueous sodium bicarbonate, and brine. The organic extracts were dried over sodium sulfate and concentrated. Purification by flash chromatography (25%. . .

DETD . . . dichloromethane (500 mL) at 0.degree. C. was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (12.3 g, 71.9 mmol). After
18 h, ethyl acetate and saturated **ammonium chloride** were added and the mixture extracted with **ammonium chloride**, sodium bicarbonate, and brine. The organic extracts were dried over sodium sulfate and concentrated. Purification by silica gel chromatography (25%. . .

DETD . . . was dried (MgSO.sub.4) and concentrated in vacuo. Flash chromatography of the residue, eluting with 5% methanol/chloroform containing a trace of **ammonium** hydroxide gave two products, The more polar product, 80 mg, 23% yield was the desired compound. MS 478 (M+). The. . .

L9 ANSWER 5 OF 144 USPATFULL

AB The invention relates to promoters of the genes glutamate dehydrogenase,
.beta.-acetylhexosaminidase and .gamma.-actin and their use in systems of expression, secretion and anti-sense of filamentary fungi. The

invention also relates to the use of the promoters of the genes which code: (I) glutamate dehydrogenase NADP depending (EC.1.4.1.4) of Penicillium chrysogenum, (II) .gamma.-N-actylhexosaminidase (EC.3.2.1.52) of Penicillium chrysogenum and (III) .gamma.-actin of Penicillium chrysogenum and Acrimonium chrysogenum, which can be used for the construction of potent vectors of expression and secretion useful both for P. chrysogenum and for A. chrysogenum and related species. These promoters can also be used for blocking the genic expression through anti-sense construction. Under the control of the above mentioned promoters, it is possible to conduct the expression of other genes in filamentary fungi, thereby increasing the production of antibiotics and/or **proteins** inherent to the same.

AN 2001:173356 USPATFULL

TI Promoters of the genes glutamate dehydrogenase .beta.-N-acetylhexosaminidase and .gamma.-actin and their use in filamentous fungi expression, secretion and antisense systems

IN Barredo Fuente, Jose Luis, Leon, Spain
Rodriguez Saiz, Marta, Pontevedra, Spain
Collados De La Vieja, Alfonso J., Leon, Spain
Moreno Valle, Migeul Angel, Leon, Spain
Salto Maldonado, Francisco, Madrid, Spain
Diez Garcia, Bruno, Cerecedo, Spain

PA Antibioticos, S.A., Leon, Spain (non-U.S. corporation)

PI US 6300095 B1 20011009
WO 9839459 19980911 <--

AI US 1999-171337 19990514 (9)
WO 1998-ES56 19980305
19990514 PCT 371 date
19990514 PCT 102(e) date

PRAI ES 1997-482 19970305

DT Utility

FS GRANTED

EXNAM Primary Examiner: Schwartzman, Robert A.

LREP Ladas & Parry

CLMN Number of Claims: 25

ECL Exemplary Claim: 1

DRWN 6 Drawing Figure(s); 6 Drawing Page(s)

LN.CNT 1084

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6300095 B1 20011009
WO 9839459 19980911 <--

AB . . . it is possible to conduct the expression of other genes in filamentary fungi, thereby increasing the production of antibiotics and/or **proteins** inherent to the same.

SUMM . . . genes in filamentous fungi can be directed under the control of the aforesaid promoters, with the production of antibiotics and/or **proteins** inherent therein being increased.

SUMM . . . genes were identified and cloned which are expressed constitutively and in which the said expression preferably does not show negative **catabolic** regulation, called hereinafter strong promoters. In general it is considered that the high-expression genes have signals in the promoter region. . .

SUMM . . . the special feature of coding for an extracellular enzyme, which allows it to be used for the expression of extracellular **proteins**.

SUMM . . . of 159 bp and 56 bp was also determined between positions 971-1130 and 1262-1318 respectively. Said ORF codes for a **protein** of 49,837 Da, with an isoelectric point of 6.18, the 461

amino acid sequence of which (SEQ ID NO:5) has. . .

SUMM . . . in position 1,324 and the TGA termination codon in position 3,112. Said ORF has no introns and codes for a **protein** of 66,545 Da, with an isoelectric point of 5.34, the 596 amino acid sequence of which (SEQ ID NO:6) has. . . the expression of the heterologous ble.sup.R gene in P. chrysogenum. In addition, the fact that the enzyme .beta.-N-acetylhexosaminidase is a **protein** abundantly secreted by P. chrysogenum to the culture medium makes it possible to use the hex gene for the expression and secretion of homologous or heterologous **proteins** in P. chrysogenum or related filamentous fungi. The genes to be expressed can be fused in a reading frame with. . .

SUMM . . . in position 494 and the TAA termination codon in position 2,250. Said ORF has 5 introns and codes for a **protein** of 41,760 Da, with an isoelectric point of 5.51, the 375 amino acid sequence of which (SEQ ID NO:7) has 98.1% identity with the amino acid sequence of the .gamma.-actin **protein** of A. nidulans. In the promoter region there are found two pyrimidine-rich zones, a presumed TATA box and four CAAT. . .

SUMM . . . in position 787 and the TAA termination codon in position 2,478. Said ORF has 5 introns and codes for a **protein** of 41,612 Da, with an isoelectric point of 5.51, the 375 amino acid sequence of which (SEQ ID NO:8) has 98.4% and 98.1% identity with the amino acid sequences corresponding to the .gamma.-actin **proteins** of A. nidulans and P. chrysogenum, respectively. In the promoter region there are found pyrimidine-rich zones and a CAAT box,. . .

SUMM . . . alone or in combination. Examples of nitrogen sources would be peptone, malt extract, yeast extract, corn steep liquor, gluten, urea, **ammonium salts**, nitrates, NZ-amine, **ammonium** sulphate, etc., used alone or in combination. Inorganic salts which can be used as components of the culture medium include. . .

SUMM . . . in the plasmid under the control of the promoter and (VII) transformant strains able to secrete homologous or heterologous extracellular **proteins** under the control of the Phex.

DETD . . . of 159 bp and 56 bp was also determined between positions 971-1130 and 1262-1318, respectively. Said ORF codes for a **protein** of 49,837 Da, with an isoelectric point of 6.18, the 461 amino acid sequence of which (SEQ ID NO:5) has. . .

DETD The presence of a major **protein** which after purification and characterization was found to be the enzyme .beta.-N-acetylhexosaminidase was determined in the P. chrysogenum mycelium obtained. . . industrial fermentation under conditions of penicillin G production. The amino acid sequence of the amino terminal end of the purified **protein** was determined by Edman's degradation method, two different sequences being obtained:

DETD . . . in position 1,324 and the TGA termination codon in position 3,112. The said ORF lacks introns and codes for a **protein** of 66,545 Da, with an isoelectric point of 5.34, the 596 amino acid sequence of which (SEQ ID NO:6) has. . .

DETD . . . Phex obtained lacked mutations and included the NcoI site above the ATG which codes for the initiator methionine of the **protein** .

DETD 2.3. Extracellular Production of **Proteins** in P. chrysogenum Using the hex Gene

DETD The enzyme .beta.-N-acetylhexosaminidase is a **protein** which is abundantly secreted by P. chrysogenum to the culture medium in industrial fermenters under conditions of penicillin G production.. . . to be secreted makes it possible to use the hex gene for the expression and secretion of homologous or heterologous **proteins**

in P. chrysogenum or related filamentous fungi.

DETD . . . region called "n", which usually has from 1 to 5 residues and is needed for the efficient translocation of the **protein** across the membrane (Met-Lys), (II) a hydrophobic region called "h", made up of 7 to 15 residues (Phe-Ala-Ser-Val-Leu-Asn-Val-Leu) (SEQ ID. . . made up of two basic residues (Lys-Arg, amino acids 97 and 98 of SEQ ID NO: 6), producing a mature **protein**.

DETD There are two possibilities when it comes to expressing and secreting **proteins** using the hex gene: (I) fusing the promoter region, including the secretion signal sequence, to the coding region of the.

.

. promoter, including the secretion sequence of the hex gene, or else the complete gene, for the expression and secretion of **proteins** of interest in P. chrysogenum or related filamentous fungi.

DETD . . . in position 2,250. Said ORF has 5 introns in positions 501-616, 649-845, 905-1046, 1078-1180 and 1953-2021 and codes for a **protein** of 41,760 Da, with an isoelectric point of 5.51, the 375 amino acid sequence of which (SEQ ID NO:7) has 98.1% identity with the amino acid sequence of the .gamma.-actin **protein** of A. nidulans. In the promoter region there are found two extensive pyrimidine-rich zones between positions 356-404 and 418-469, a. . .

DETD . . . PactPc obtained lacked mutations and included the NcoI site above the ATG which codes for the initiator methionine of the **protein**.

DETD . . . in position 2,478. Said ORF has 5 introns in positions 794-920, 952-1,123, 1,180-1,289, 1,321-1,410 and 2,183-2,249 and codes for a **protein** of 41,612 Da, with an isoelectric point of 5.51, the 375 amino acid sequence of which (SEQ ID NO:8) has 98.4% and 98.1% identity with the amino acid sequences of the .gamma.-actin **proteins** of A. nidulans and P. chrysogenum, respectively. In the promoter region there is found a pyrimidine-rich zone between positions 607-654,. . .

DETD . . . fact that the act gene has an NcoI site above the ATG which codes for the initiator methionine of the **protein**. To this end the ble.sup.R gene was subcloned in the plasmid pALCact1 (carrying the PactAc) previously digested with NcoI-ApaI, giving. . .

L9 ANSWER 6 OF 144 USPATFULL

AB This invention relates to the identification of homologs of atrazine chlorohydrolase and the use of these homologs to degrade s-triazine-containing compounds. In particular, this invention includes the identification of homologs of atrazine chlorohydrolase encoded by a DNA fragment having at least 95% homology to the sequence from the nucleic acid sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1, where the DNA fragment is capable of hybridizing under stringent conditions to SEQ ID NO:1 and has altered catalytic activity as compared with wild-type atrazine chlorohydrolase.

AN 2001:116807 USPATFULL

TI DNA molecules and **protein** displaying improved triazine compound degrading ability

IN Wackett, Lawrence P., St. Paul, MN, United States
Sadowsky, Michael J., Roseville, MN, United States
de Souza, Mervyn L., St. Paul, MN, United States
Minshull, Jeremy S., Menlo Park, CA, United States

PA Regents of the University of Minnesota, Minneapolis, MN, United States (U.S. corporation)
Maxygen Inc., Redwood City, CA, United States (U.S. corporation)

PI US 6265201 B1 20010724
WO 9831816 19980723

AI   US 1998-155036   19980917 (9)
        WO 1998-US944   19980116
                                19980917 PCT 371 date
                                19980917 PCT 102(e) date
PRAI   US 1997-35404P   19970117 (60)
DT   Utility
FS   GRANTED
EXNAM   Primary Examiner: Prouty, Rebecca E.; Assistant Examiner: Hutson, Richard
LREP   Mueting, Raasch & Gebhardt, P.A.
CLMN   Number of Claims: 10
ECL   Exemplary Claim: 1
DRWN   18 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 1381
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI   DNA molecules and **protein** displaying improved triazine compound degrading ability
PI   US 6265201   B1   20010724
        WO 9831816 19980723

SUMM   . . . isolated from a Pseudomonas sp. strain. See, for example, de Souza et al., Appl. Environ. Microbiol., 61, 3373 (1995). The **protein** expressed by the gene disclosed by de Souza et al., degrades atrazine, for example, at a V.sub.max of about 2.6 .mu.mol of hydroxyatrazine per min per mg **protein**. Although this is significant, it is desirable to obtain genes and the **proteins** they express that are able to dechlorinate triazine-containing compounds with chlorine moieties at an even higher rate and/or under a. . . in which the wild type enzyme is not stable, efficient, or active. Similarly, it is desirable to obtain genes and **proteins** encoded by these genes that degrade triazine-containing compounds such as those triazine containing compounds available under the trade names; "AMETRYN", "PROMETRYN", "CYANAZINE", "MELAMINE", "SIMAZINE", as well as TERBUTHYLAZINE and desethyldesisopiopylatriazine. It is also desirable to identify **proteins** expressed in organisms that degrade triazine-containing compounds in the presence of other nitrogen sources such as ammonia and nitrate.

SUMM   . . . salt concentration, pH, improved activity in a soil environment, and the like, as compared with the wild-type atrazine chlorohydrolase (AtzA) **protein**.

SUMM   The invention also relates to s-triazine-degrading **proteins** hlaving at least one amino acid different from the **protein** of SEQ ID NO:2, wherein the coding region of the nucleic acid encoding the s-triazine degrading **protein** has at least 95% homology to SEQ ID NO:1 and wherein the s-triazine-degrading **protein** has an altered catalytic activity as compared with the **protein** having the sequence of SEQ ID NO:2. In one embodiment, the **protein** is selected from the group consisting of SEQ ID NOS:5, 6 and 22-26. In one embodiment the substrate for the s-triazine degrading **protein** is ATRAZINE. In another embodiment the substrate for the s-triazine degrading **protein** is TERBUTHYLAZINE and in yet another embodiment the substrate for the s-triazine degrading **protein** is MELAMINE. In another embodiment this invention relates to a remediation composition comprising a cell producing at least one s-triazine-degrading **protein** having at least one amino acid different from the **protein** of SEQ ID NO:2, wherein the coding region of the nucleic acid encoding the s-triazine degrading **protein** has at least 95% homology to SEQ ID NO:1 and wherein the s-triazine-degrading **protein** has an altered catalytic activity as compared with the **protein** having the sequence of SEQ ID

NO:2. In a preferred embodiment the composition is suitable for treating soil or water. In another embodiment the remediation composition comprises at least one s-triazine-degrading **protein** having at least one amino acid different from the **protein** of SEQ ID NO:2, wherein the coding region of the nucleic acid encoding the s-triazine degrading **protein** has at least 95% homology to SEQ ID NO:1 and wherein the s-triazine-degrading **protein** has an altered catalytic activity as compared with the **protein** having the sequence of SEQ ID NO:2. In a preferred embodiment this composition is also suitable for treating soil or water. In one embodiment the remediation composition comprises the **protein** bound to an immobilization support. In yet another embodiment, these **proteins** are homotetramers, such as the homotetramers formed by AtzA.

SUMM In another embodiment the invention relates to a **protein** selected from the group consisting of **proteins** comprising the amino acid sequences of SEQ ID NOS:5, 6 and 22-26.

SUMM . . . hybridizing under stringent conditions to SEQ ID NO:1 and wherein there is at least one amino acid change in the **protein** encoded by the DNA fragment as compared with SEQ ID NO:2 and wherein the **protein** encoded by the DNA fragment is capable of dechlorinating at least one s-triazine-containing compound and has a catalytic activity different from the enzymatic activity of the **protein** of SEQ ID NO:2. In one embodiment the s-triazine-containing compound is ATRAZINE, TERBUTHYLAZINE, or MELAMINE. In one embodiment.

SUMM . . . to a method for treating a sample comprising an s-triazine containing compound comprising the step of adding a adding a **protein** to a sample comprising an s-triazine-containing compound wherein the **protein** is encoded by gene having at least a portion of the nucleic acid sequence of the gene having at least. . . of hybridizing under stringent conditions to SEQ ID NO:1, wherein there is at least one amino acid change in the **protein** encoded by the DNA fragment as compared with SEQ ID NO:2 and wherein the **protein** has an altered catalytic activity as compared to the **protein** having the amino acid sequence of SEQ ID NO:2. In one embodiment, the composition comprises bacteria expressing the **protein**. In one embodiment the s-triazine-containing compound is atrazine, in another the s-triazine-containing compound is TERBUTHYLAZINE and in another the s-triazine containing compound is (2,4,6-triamino-s-triazine). In one embodiment, the **protein** encoded by the gene is selected from the group consisting of SEQ ID NOS:5, 6 and 22-26.

SUMM . . . acid sequence encoding atrazine chlorohydrolase, mutagenizing the nucleic acid to obtain a modified nucleic acid sequence that encodes for a **protein** having an amino acid sequence with at least one amino acid change relative to the amino acid sequence of the atrazine chlorohydrolase, screening the **proteins** encoded by the modified nucleic acid sequence; and selecting **proteins** with altered catalytic activity as compared to the catalytic activity of the atrazine chlorohydrolase. Preferably, the atrazine chlorohydrolase nucleic acid. . .

DETD The present invention provides isolated and purified DNA molecules, and isolated and purified **proteins**, involved in the degradation of s-triazine-containing compounds. The **proteins** encoded by the genes of this invention are involved in the dechlorination and/or the deamination of s-triazine-containing, compounds. The wild type AtzA

protein can catalyze the dechlorinationi of s-triazine-containing compounds but not the deamination of these compounds. The dechlorination reaction occurs on s-triazine. . .

DETD . . . of the genome of Pseudomonas sp. ADP (ADP is strain designiation for Atrazine-degrading Pseudomonas) bacterium. Specifically, these genomic fragments encode **proteins** involved in s-triazine dechlorination. The rate of degradation of atrazine that results from the expression of these fragments in E.. . . native Pseudomonas sp. strain ADP, this degradation in E. coli is unaffected by the presence of inorganic nitrogen sources like **ammonium chloride**. This is particularly advantageous for regions contaminated with nitrogen-containing fertilizers or herbicides, for example. The expression of atrazine degradation activity. . .

DETD As used herein, the gene encoding a **protein** capable of dechlorinating atrazine and originally identified in Pseudomonas sp. strain ADP and expressed in E. coli is referred to as "atzA", whereas the **protein** that it encodes is referred to as "AtzA." Examples of the cloned wild type gene sequence and the amino acid. . . are provided as SEQ ID NO:1 and SEQ ID NO:2 respectively. As also used herein, the terms atrazine chlorohydrolase (AtzA) **protein**, atrazine chlorohydrolase enzyme, or simply atrazine chlorohydrolase, are used interchangeably, and refer to an atrazine chlorohydrolase enzyme involved in the. . .

DETD A "homolog" of atrazine chlorohydrolase is an enzyme derived from the gene sequence encoding atrazine chlorohydrolase where the **protein** sequence encoded by the gene is modified by amino acid deletion, addition, substitution, or truncation but that nonetheless is capable. . . chlorohydrolase (AtzA) has a nucleic acid sequence that is different from the atzA sequence (SEQ ID NO:1) and produces a **protein** with modified biological properties or, as used herein, "altered enzymatic activities." These homologs include those with altered catalytic rates as. . . salt concentration, pH, improved activity in a soil environment. and the like, as compared with the wild-type atrazine chlorolhydrolase (AtzA) **protein**. Thus, provided that two molecules possess enzymatic activity to an s-triazine-containing substrate and one molecule has the gene sequence of. . . of the homolog differs from SEQ ID NO:1 such that there is at least one amino acid change in the **protein** encoded by SEQ ID NO:1 (i.e., SEQ ID NO:2); 2) the homolog has different enzymatic characteristics from the **protein** encoded by SEQ ID NO:1 such as, but not limited to, an altered substrate preference, altered rate of activity, or. . . or the like, as discussed supra; and 3) where the coding region of the nucleic acid sequence encoding the variant **protein** has at least 95% homology to SEQ ID NO:1.

DETD As used herein, the terms "isolated and purified" refer to the isolation of a DNA molecule or **protein** from its natural cellular environment, and from association with other coding regions of the bacterial genome, so that it can. . .

DETD . . . i.e. recently evolved gene. That the gene is recently evolved is supported by the attributes of the gene and the **protein** encoded by the gene. For example: (i) the gene has a limited s-triazine range that includes atrazine and the structurally. . . act on all s-triazines; (ii) the gene has a high sequence homology to genes isolated from other bacteria that produce **proteins** with atrazine-degrading activity; (iii) is not organized with the atzB and

atzC genes in a contiguous arrangement such as an. . .

DETD . . . from a survey of atrazine-degrading bacteria are so structurally and catalytically similar suggest that the atza gene and the AtzA **protein** can be improved and will be improved naturally over time. Indeed, most biodegradative enzymes are orders of magnitude below diffusion limiting enzyme rates and, under this hypothesis, are also candidates for gene and **protein** modifications.

DETD . . . embodiment of this invention, a method is disclosed for selecting or screening modified and improved atzA gene sequences that encode **protein** with improved enzymatic activity, whether the activity is enzymatic rate, using atrazine as a substrate, as compared to the wild-type. . . to obtain altered atzA sequences, selecting or screening for clones expressing altered AtzA activity and selecting gene sequences encoding AtzA **protein** with improved s-triazine-degrading activity.

DETD There are a number of methods in use for creating mutant **proteins** in a library format from a parent sequence. These include the polymerase chain reaction (Leung, D. W. et al. Technique. . . et al., Gene 44:177-183 (1986), Hermes, J. D. et al., Proc. Natl. Acad. Sci. USA 87:696-700 (1990), Delgrave et al. **Protein** Engineerinn 6:327-331, (1993), Delgrave et al. Bio/Technology 1:1548-1552 (1993), and Goldman, E R et al., Bio/Technology 10: 1557-1561 (1992)), as. . .

DETD Once intact gene sequences are reassembled, they are incorporated into a vector suitable for expressing **protein** encoded by the reassembled nucleic acid, or as provided in Example 1, where the gene sequences are already in a. . . The host, generally an E. coli species, is used in assays to screen or select for clones expressing the AtzA **protein** under defined conditions. The type of organism can be matched to the mutagenesis procedure and in Example 2, a preferred. . .

DETD . . . assays suitable for use in this invention can take any of a variety of forms for determining whether a particular **protein** produced by the organism containing the variant atzA sequences expresses an enzyme capable of dechlorinating or deaminating s-triazine compounds. Therefore,. . .

DETD . . . can be altered to a pH range of about 5 to about 9. These assays would likely use isolated homolog **protein** to permit an accurate assessment of the effect of pH. The assay, or a modification of the assay, suitable for. . .

DETD . . . rule out if the apparent enhanced activity of the enzyme is the result of a faster or more efficient AtzA **protein** production or whether the effect observed is the result of an altered atzA gene sequence. For example, in Example 2,. . .

DETD . . . homologs are isolated for further analysis. Clones containing putative faster enzyme(s) can be picked, grown in liquid culture, and the **protein** homolog can be purified, for example, as described (de Souza et al. J. Bacteriology, 178:4894-4900 (1996)). The genes encoding the. . . as known in the art, for extracellular expression or the homologs can be purified from bacteria. An exemplary method for **protein** purification is provided in Example 4. In a preferred

method, **protein** was collected from bacteria using **ammonium** sulfate precipitation and further purified by HPLC (see for example, de Souza et al., App. Envir. Microbio. 61:3373-3378 (1995)).

DETD . . . found to have at least a 10 fold higher activity and contained 8 different amino acids than the native AtzA **protein** (A7 and T7, see FIGS. 1-4). A subsequent round of DNA shuffling starting with the homolog gene sequence yielded further. . . enzyme and other AtzA homologs (clones A40, A42, A44, A46, A60 corresponding to nucleic acid SEQ ID NOS:17-21 and to **protein** SEQ ID NOS:22-26, respectively) represent **catabolic** enzymes modified in their biological activity. Preferred homologs identified in initial studies include A7, T7, A11, A44, and A46.

DETD . . . and the kinetic improvement of the homologs has important implications for enzymatic environmental remediation of this widely used herbicide. Less **protein** is required to dechlorinate the same amount of atrazine. Importantly, the **protein** can also be used for degradation of the s-triazine-compound TERBUTHYLAZINE.

DETD This invention also relates to nucleic acid and **protein** sequences identified from the homologs of this invention. Peptide and nucleic acid fragments of these sequences are also contemplated and. . . of this invention. The homologs of this invention include those with an activity different from the native atrazine chlorohydrolase (AtzA) **protein**. As noted stipra. an activity that is different from the native atrazine chlorohydrolase **protein** includes enzymatic activity that is improved or is capable of functioning under different conditions such as salt concentration, temperature, altered. . . the homologs hybridize to a DNA molecule complementary to the wild-type coding region of a DNA molecule encoding wild-type AtzA **protein**, such as the sequence provided in SEQ ID NO:1, under high to moderate stringency hybridization conditions. The homologs preferably have. . .

DETD . . . ID NO:4. FIG. 3 provides the amino acid sequence alignment of SEQ ID NO:2, the amino acid sequence of the **protein** encoded by SEQ ID NO:1, with SEQ ID NO:5 and FIG. 4 provides the amino acid sequence alignment of SEQ. . .

DETD . . . this invention, the success attributed to the identification of homologs of AtzA may be based on the recognition that this **protein** is not evolutionarily mature. Therefore, not all gene sequences are good candidates as the starting material for identifying a number of biological variants of a particular **protein** and similarly, not all enzymes are amenable to the order of magnitude of rate enhancement by directed evolution using DNA. . .

DETD . . . selected for expression, and will be apparent to those skilled in the art. Induction of cells to express the AtzA **protein** is accomplished using the procedures required by the particular expression system selected. The host cells referred to in this disclosure. . .

DETD This invention also relates to isolated **proteins** that are the product of the gene sequences of this invention. The isolated **proteins** are **protein** homologs of the wild-type atrazine chlorohydrolase enzyme despite their potential for altered substrate preference. The **protein** can be isolated in a variety of methods disclosed in the art and a preferred method for isolating the **protein** is provided in Examples 4 and 5 and in the publications of de Souza et al. (supra).

DETD The wild-type AtzA **protein** acts on Atrazine, desethylatrazine,

Desisopropylatrazine and SIMAZINE but did not degrade Desethyldesisopropylatrazine or MELAMINE and only poorly degraded TERBUTHYLAZINE. Homologs identified in this invention have a spectrum of
substrate preferences identical to the wild-type AtzA **protein** and in addition, for example, are able to degrade other substrates such as TERBUTHYLAZINE. That homologs were identified that were. . . used on the wild-type progenitor atzA gene or on the homologs produced by this invention to produce even more useful **proteins** for environmental remediation of s-triazine-containing compounds. Example 7 provides an assay for detecting degradation, including deamination, of a
soluble s-triazine-containing. . .

DETD Various environmental remediation techniques are known that utilize high
levels of **proteins**. Bacteria or other hosts expressing the homologs of this invention can be added to a remediation mix or mixture in need of remediation to promote contaminate degradation. Alternatively, isolated AtzA homologs can be added. **Proteins** can be bound to immobilization supports, such as beads, particles, films, etc., made from latex, polymers, alginate, polyurethane, plastic,
glass, polystyrene, and other natural and man-made support materials. Such immobilized **protein** can be used in packed-bed columns for treating water effluents. The **protein** can be used to remediate liquid samples, such as contaminated water, or solids. The advantage of some of the homologs. . .

DETD . . . DNA fragments. The GCG sequence analysis software package (Genetics Computer Group, Inc., Madison, Wis.) was used for all DNA and **protein** sequence comparisons. Radiolabelled chemicals were obtained from Ciba Geigy Corp., Greensboro, N.C.

DETD **Protein** Purification of AtzA or Homologs

DETD . . . type atzA gene or alternatively with a homolog, in a vector capable of directing expression of the gene as a **protein**, was grown overnight at 37.degree. C. in eight liters of LB medium containing
25 .mu.g/ml chloramphenicol. The culture medium was. . .

DETD Where purified **protein** was desired, the solution was loaded onto a Mono Q HR 16/10 Column (Pharmacia LKB Biotechnology, Uppsala, Sweden). The column was washed with 25 mM MOPS buffer (pH 6.9), and the **protein** was eluted with a 0-0.5 M KCl gradient. **Protein** eluting from the column was monitored at 280 nm by using a Pharmacia U.V. **protein** detector. Pooled fractions containing the major peak were dialyzed overnight against 1 liter 25 mM MOPS buffer (pH 6.9).
The. . .

DETD **Protein** Verification: **Protein** subunit sizes were determined by SDS polyacrylamide gel electrophoresis by comparison to known standard **proteins**, using a Mini-Protean II gel apparatus (Biorad, Hercules, Calif.). The size of the holoenzyme was determined by
gel filtration chromatography on a Superose 6 HR (1.0.times.30.0 cm) column, using an FPLC System (Pharmacia, Uppsala, Sweden). The **protein** was eluted with 25 mM MOPS buffer (pH 6.9) containing 0.1 M NaCl. **Proteins** with known molecular weights were used as chromatography standards. Isoelectric point determinations were done using a Pharmacia Phast-Gel System and. . .

DETD Enzyme Kinetics. Purified AtzA **protein** and homologs of the **protein** at 50 .mu.g/ml, were separately added to 500 .mu.l of different concentrations of atrazine (23.3 .mu.M, 43.0 .mu.M, 93 .mu.M,.

. .

DETD . . . degradation. From experiments done with Pseudomonas species strain ADP on solid media with 500 ppm atrazine and varying concentrations of **ammonium chloride**, **ammonium chloride** concentrations as low as 0.6-1.2 mM were sufficient to inhibit visible clearing on the plates, even after 2 weeks of. . . using E. coli DH5.alpha. (pMD1 or pMD2) and other E. coli strains, atrazine degradation was observed in the presence of **ammonium chloride** concentrations as high as 48 mM. This value is almost 40-80 fold higher than the wild-type tolerance for **ammonium chloride** with concomitant atrazine degradation. Therefore, it was not necessary to use media free of exogenous ammonia in the screening assays.

DETD . . . of TERBUTHYLAZINE degradation. Sample 1 is a control sample without enzyme. Sample 2 uses a two fold excess of AtzA **protein** as compared to the concentration of homolog added in Sample 3 and Sample 4. Sample 3 employed the T7 homolog. . . .

DETD . . . Example 2 are subjected to further mutagenesis and colonies capable of growing in MELAMINE can be identified. Colonies containing the **protein** AtzA are tested for growth in MELAMINE under identical conditions. Other s-triazine containing compounds such as the pesticides available under. . .

L9 ANSWER 7 OF 144 USPATFULL

AB This invention is directed to compounds of the formula ##STR1##

and the pharmaceutically-acceptable salts thereof, where the substituents are as defined in the Specification, which are growth hormone secretogogues and which increase the level of endogenous growth hormone. The compounds of this invention are useful for the treatment and prevention of osteoporosis and/or frailty, congestive heart failure, frailty associated with aging, obesity; accelerating bone fracture repair, attenuating **protein catabolic** response after a major operation, reducing cachexia and **protein** loss due to chronic illness, accelerating wound healing, or accelerating the recovery of burn patients or patients having undergone major surgery; improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis. The compounds of the present invention are also useful in treating osteoporosis and/or frailty when used in combination with: a bisphosphonate compound such as alendronate; estrogen, premarin, and optionally progesterone; an estrogen agonist or antagonist; or calcitonin, and pharmaceutical compositions useful therefor. Further, the present invention is directed to pharmaceutical compositions useful for increasing the endogenous production or release of growth hormone in a human or other animal which comprises an effective amount of a compound of the present invention and a growth hormone secretagogue selected from GHRP-6, Hexarelin, GHRP-1, growth hormone releasing factor (GRF), IGF-1, IGF-2 or B-HT920. The invention is also directed to intermediates useful in the preparation of compounds of Formula I.

AN 2001:97924 USPATFULL

TI Dipeptide derivatives as growth hormone secretagogues

IN Carpino, Philip Albert, Groton, CT, United States

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PA   Pfizer Inc., New York, NY, United States (U.S. corporation)
PI   US 6251902   B1   20010626
        WO 9858947 19981230   <--
AI   US 1999-380887   19990908 (9)
        WO 1998-IB873   19980605
                                19990908   PCT 371 date
                                19990908   PCT 102(e) date
PRAI   US 1997-50764P   19970625 (60)
DT   Utility
FS   GRANTED
EXNAM   Primary Examiner: Raymond, Richard L.
LREP   Richardson, Peter C., Benson, Gregg C., Ronau, Robert T.
CLMN   Number of Claims: 50
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 6506
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 6251902   B1   20010626
        WO 9858947 19981230   <--
AB   . . . treatment and prevention of osteoporosis and/or frailty, congestive heart failure, frailty associated with aging, obesity; accelerating bone fracture repair, attenuating **protein catabolic** response after a major operation, reducing cachexia and **protein** loss due to chronic illness, accelerating wound healing, or accelerating the recovery of burn patients or patients having undergone major surgery; improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis. The compounds of the present invention are also useful. . .
SUMM   1. Increased rate of **protein** synthesis in substantially all cells of the body;
SUMM   . . . in lean body mass and concomitant increase in total body fat, particularly in the truncal region. Decreased skeletal and cardiac **muscle** mass and **muscle** strength lead to a significant reduction in exercise capacity. Bone density is also reduced. Administration of exogenous growth hormone has. . .
SUMM   . . . D., et al, Horm Res 36 (Suppl 1):73 (1991)) has been shown to produce increases in lean body, hepatic and **muscle** mass while decreasing fat mass. Thus, GH therapy for obesity would seem attractive except for the diabetogenic effects of GH.
SUMM   methods for accelerating bone fracture repair, attenuating **protein catabolic** response after a major operation, reducing cachexia and **protein** loss due to chronic illness such as AIDS or cancer, accelerating would healing, or accelerating the recovery of burn patients. . .
SUMM   methods for improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis, which methods comprise administering to a human or other. . .
SUMM   methods for increasing piglet number, increasing pregnancy rate in sows, increasing viability of piglets, increasing weight of piglets or increasing **muscle** fiber size in piglets which comprise administering to a sow or piglet an effective amount of a compound of Formula. . .
SUMM   methods for increasing **muscle** mass, which comprise administering to a human or other animal such as dogs, cats, horses, cattle, pigs, chickens, turkeys, sheep. . .
SUMM   In yet another aspect, this invention provides methods for improving

muscle strength, mobility, maintenance of skin thickness, metabolic homeostasis and renal homeostasis, which comprise administering to a human or other animal. . .

SUMM . . . arylacetyl and .alpha.-aminoacyl, or .alpha.-aminoacyl-.alpha.-aminoacyl wherein said .alpha.-aminoacyl moieties are independently any of the naturally occurring L-amino acids found in **proteins**,

SUMM . . . cats, camels and horses; treating growth hormone deficient adult humans or other animals especially dogs, cats, camels and horses; preventing **catabolic** side effects of glucocorticoids, treating osteoporosis, stimulating the immune system, accelerating wound healing, accelerating bone fracture repair, treating growth retardation,. . . osteochondrodysplasias, Noonans syndrome, sleep disorders, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treating pulmonary dysfunction and ventilator dependency; attenuating **protein catabolic** response after a major operation; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treating hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to prevent. . . of thymic function; adjunctive therapy for patients on chronic hemodialysis; treating immunosuppressed patients and enhancing antibody response following vaccination; improving **muscle** strength, increasing **muscle** mass, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulating osteoblasts, bone remodeling, and cartilage. . .

SUMM . . . stimulation of pre- and post- natal growth, enhanced feed efficiency in animals raised for meat production, improved carcass quality (increased **muscle** to fat ratio) (Campbell, R. G. et al., (1989), J. Anim. Sci. 67, 1265; Dave, D. J., Bane, D. P.,. . . antibody response following vaccination or improved developmental processes; and may have utility in aquaculture to accelerate growth and improve the **protein**-to-fat ratio in fish.

SUMM . . . 14-22; Mankin. J. J. et al., J. of Bone and Joint Surgery, Vol. 60-A, #8, Dec. 1978, pp. 1071-1075); attenuating **protein catabolic** response after major surgery, accelerating recovery from burn injuries and major surgeries such as gastrointestinal surgery; stimulating the immune system. . . heart failure, treating acute or chronic renal failure or insufficiency, treating obesity; treating growth retardation, skeletal dysplasia and osteochondrodysplasias; preventing **catabolic** side effects of glucocorticoids; treating Cushing's syndrome; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer; accelerating weight gain and **protein** accretion in animals receiving total parenteral nutrition; providing adjuvant treatment for ovulation induction and to prevent gastrointestinal ulcers; improving **muscle** mass, strength and mobility; maintenance of skin thickness, and improving vital organic function and metabolic homeostasis.

SUMM . . . hereby incorporated by reference. In another aspect, this invention provides methods for accelerating bone fracture repair and wound healing, attenuating **protein catabolic** response after a major operation, and reducing cachexia and **protein** loss due to chronic illness, which comprise administering to a human or another animal, especially dogs, cats and horses in. . .

SUMM . . . dissolution, chloroform or methanol was employed. Thermospray
mass spectra (TSMS) were obtained on a Trio-1000 by Fisions
spectrometer
using 0.1M **ammonium** acetate in 1/4 water/methanol. The
protonated parent ion is reported as (M+1).sup.+. For initial sample
dissolution chloroform or methanol were. . . visualized (after
elution with the indicated solvent(s)) by UV, iodine or by staining
with
15% ethanolic phosphomolybdic acid or ceric sulfate/**ammonium**
molybdate and heating on a hot plate. The terms "concentrated" and
"coevaporated" refer to removal of solvent at water aspirator. . .

DETD . . . the filtrate was concentrated in vacuo to give 180 g of a
clear
oil. Purification by silica gel chromatography using **ammonium**
hydroxide/methanol/chloroform (0.25:5:95) as eluent yielded the title
compound of part 2-F as a clear oil (102 mg, 65%): +APcl MS. . .

DETD . . . reaction was quenched with methanol and concentrated in vacuo.
Ethyl acetate was added, and the mixture was extracted with saturated
ammonium chloride solution, brine, dried over
anhydrous magnesium sulfate, filtered and concentrated in vacuo to give
crude product. Purification by silica gel. . .

DETD . . . temperature and stirred for about 2.5 hours. The reaction
mixture was cooled to about 0.degree. C., and quenched with saturated
ammonium chloride solution. The mixture was then
diluted with ethyl acetate, and washed three times with saturated
sodium
bicarbonate solution, twice with. . .

DETD . . . 0.0222 mmol) portionwise. The reaction was stirred for about 3
hours at room temperature. The reaction was quenched with saturated
ammonium chloride solution, the methanol was removed
in vacuo, and the aqueous mixture was extracted several times with
ethyl
acetate. The combined. . .

L9 ANSWER 8 OF 144 USPATFULL

AB Methods for treating non-insulin-taking Type II diabetes mellitus which
comprise administering a therapeutically effective amount of an amylin
agonist.

AN 2000:150138 USPATFULL

TI Treatment of Type II diabetes mellutis with amylin agonists

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Thompson, Robert G., San Diego, CA, United States
Mullane, John F., Cardiff, CA, United States

PA Amylin Pharmaceuticals, Inc., San Diego, CA, United States (U.S.
corporation)

PI US 6143718 20001107 <--

AI US 1995-483188 19950607 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Woodward, Michael P.; Assistant Examiner: Mohamed,
Abdel A.

LREP Lyon & Lyon LLP

CLMN Number of Claims: 21

ECL Exemplary Claim: 1

DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 1012

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6143718 20001107 <--

SUMM Amylin is a 37 amino acid **protein** hormone. It was isolated,
purified and chemically characterized as the major component of amyloid

deposits in the islets of pancreases. . . .

SUMM The first discovered action of amylin was the reduction of insulin-stimulated incorporation of glucose into glycogen in rat skeletal **muscle** (Leighton et al., Nature 335:632-635 (1988)); the **muscle** was made "insulin-resistant". Subsequent work with rat soleus **muscle** ex-vivo and in vitro has indicated that amylin reduces glycogen-synthase activity, promotes conversion of glycogen phosphorylase from the inactive b. . .

SUMM . . . Diabetologia 35:116-120 (1992)). Thus, amylin could act there as an anabolic partner to insulin in liver, in contrast to its **catabolic** action in **muscle**.

SUMM In fat cells, contrary to its action in **muscle**, amylin has no detectable actions on insulin-stimulated glucose uptake, incorporation of glucose into triglyceride, CO.sub.2 production (Cooper et al., Proc.. . . Nutrition and Metabolism--Clinical and Experimental, Vol. 6(1), pages 13-18 (1993)). Amylin thus exerts tissue-specific effects, with direct action on skeletal **muscle**, marked indirect (via supply of substrate) and perhaps direct effects on liver, while adipocytes appear "blind" to the presence or. . .

DETD Amylin agonist agents may be identified by activity in the receptor binding and soleus **muscle** assays described below. Amylin agonist activity of compounds may also be assessed by the ability to induce hypercalcemia and/or hyperglycemia. . .

DETD . . . binding assay can identify both candidate amylin agonists and antagonists and can be used to evaluate binding, while the soleus **muscle** assay can be used to distinguish between amylin agonists and antagonists. Preferably, agonist compounds exhibit activity in the receptor binding. . . to 5 nM, preferably less than about 1 nM and more preferably less than about 50 pM. In the soleus **muscle** assay these compounds preferably show EC.sub.50 values on the order of less than about 1 to 10 micromolar.

DETD Assays of biological activity of amylin agonists, including amylin agonist analogue preparations, in the soleus **muscle** are performed using previously described methods (Leighton, B. and Cooper, G. J. S., Nature, 335:632-635 (1988); Cooper, G. J. S.,. . . USA 85:7763-7766 (1988)). In summary, amylin agonist activity is assessed by measuring the inhibition of insulin-stimulated glycogen synthesis in soleus **muscle**. Amylin antagonist activity is assessed by measuring the resumption of insulin-stimulated glycogen synthesis in the presence of 100 nM rat. . . to compete with amylin in the receptor binding assay. These compounds have negligible antagonist activity as measured by the soleus **muscle** assay and were shown to act as amylin agonists.

DETD . . . acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid and camphorsulfonic acid. Salts prepared with bases include **ammonium salts**, alkali metal salts, e.g. sodium and potassium salts, and alkali earth salts, e.g. calcium and magnesium salts. Acetate, hydrochloride, and. . .

DETD . . . e.g., Remington's Pharmaceutical Sciences by E. W. Martin. See also Wang, Y. J. and Hanson, M. A. "Parenteral Formulations of **Proteins** and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988).

DETD . . . oral supplement test meal is a commercially available nutritional supplement which contains 1.01 kcal/ml with the caloric

composition being 24% **protein**, 21% fat and 55% carbohydrate. Sodium and calcium caseinates along with soy **protein** provide the **protein** calories; partially hydrogenated soybean oil provides the fat calories; and sucrose and corn syrup provide the carbohydrate calories.

L9 ANSWER 9 OF 144 USPATFULL

AB The present invention concerns new lipid derivatives of phosphonocarboxylic acids of the general formula I, ##STR1## in which the meaning of the symbols is elucidated in the description, tautomers thereof and their physiologically tolerated esters and salts of inorganic or organic bases as well as processes for the production thereof and pharmaceutical agents containing these compounds.

AN 2000:142364 USPATFULL

TI Phospholipid derivatives of phosphono-carboxylic acids, the production of said derivatives and the use of said derivatives as antiviral medicaments

IN Zilch, Harald, Alsenweg 24, D-68305 Mannheim, Germany, Federal Republic of
Herrmann, Dieter, Bothestrasse 54/1, D-69126 Heidelberg, Germany, Federal Republic of
Opitz, Hans-George, Im Netztal 46, D-69469 Weinheim, Germany, Federal Republic of
Zimmermann, Gerd, Dornheimer Ring 4, D-68309 Mannheim, Germany, Federal Republic of

PI	US 6136797	20001024		<--
	WO 9722613 19970626			<--
AI	US 1998-77891	19980827 (9)		
	WO 1996-EP5647	19961216		
		19980827	PCT 371 date	
		19980827	PCT 102(e) date	
PRAI	DE 1995-19547023	19951215		
	DE 1996-19643416	19961022		

DT Utility
FS Granted
EXNAM Primary Examiner: Ambrose, Michael G.
LREP Arent Fox Kintner Plotkin Kohn
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 796
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI	US 6136797	20001024	<--
	WO 9722613 19970626		<--

SUMM In order to improve **catabolic** stability, nucleosides such as e.g. ara-C and ara-A have been chemically bound to phospholipids. The corresponding derivatives exhibited less toxicity. . . .

SUMM In addition the in vivo distribution is improved by a better binding of the conjugate to plasma and tissue **proteins**. The conjugate is primarily oxidized by normal biotransformation from a thioether (n=0) to a sulfoxide (n=1) which, however, due to. . .

SUMM Alkali, alkaline-earth and **ammonium salts** of the carboxyl and phosphonate group come above all into consideration as possible salts of the compounds of the general. . . potassium salts are preferred as the alkali salts. Magnesium and calcium salts come in particular into consideration as alkaline-earth salts. **Ammonium salts** are understood according to the invention as salts which contain the **ammonium** ion that can be substituted up to four times by alkyl residues with 1-4 carbon atoms and/or by aralkyl

residues. . .

L9 ANSWER 10 OF 144 USPATFULL
AB Compounds of peptide mimetic nature having the general formula I ##STR1## wherein a and b are independently 1 or 2, R.sup.1 and R.sup.2 are independently H or C.sub.1-6 alkyl, G and J are independently, inter alia, aromats, and D and E are independently several different groups are growth hormone secretagogous with improved bioavailability.
AN 2000:131863 USPATFULL
TI Compounds with growth hormone releasing properties
IN Hansen, Thomas Kruse, Herlev, Denmark
Peschke, Bernd, Maaloev, Denmark
Lau, Jesper, Farum, Denmark
Lundt, Behrend Friedrich, Kokkedal, Denmark
Ankersen, Michael, Frederiksberg, Denmark
Watson, Brett, Vaerloese, Denmark
Madsen, Kjeld, Vaerloese, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 6127391 20001003 <--
AI US 1998-218686 19981221 (9)
RLI Division of Ser. No. US 1996-769020, filed on 18 Dec 1996
PRAI DK 1995-1462 19951222
DK 1996-698 19960625
DK 1996-812 19960724
DK 1996-1248 19961106
US 1996-22062P 19960722 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Aulakh, Charanjit S.
LREP Zelson, Steve T., Rozek, Carol E.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 8344
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6127391 20001003 <--
SUMM . . . of growing. In addition, growth hormone is known to have a number of effects on metabolic processes, e.g., stimulation of **protein** synthesis and free fatty acid mobilisation and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism.. . .
SUMM In disorders or conditions where increased levels of growth hormone is desired, the **protein** nature of growth hormone makes anything but parenteral administration non-viable. Furthermore, other directly acting natural secretagogues, e.g., GHRH and PACAP,. . .
SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids, prevention and treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating borte. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation, treatment of pulmonary dysfunction and ventilator dependency, attenuation of **protein** **catabolic** responses after major surgery, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis, adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function, treatment of immunosuppressed patients, improvement in **muscle** strength,

mobility, maintenance of skin thickness, metabolic homeostasis, renal homeostasis in the frail elderly, stimulation of osteoblasts, bone remodelling and. . .

DETD . . . at 1 mL/min at 42.degree. C. The column was equilibrated with 5% acetonitrile in a buffer consisting of 0.1 M **ammonium** sulfate, which was adjusted to pH 2.5 with 4M sulfuric acid, after injection the sample was eluted by a gradient. . .

DETD . . . mixture was stirred for 1.5 h at -78.degree. C. and then warmed to room temperature. A 10% aqueous solution of **ammonium chloride** (200 ml) was added dropwise. The phases were separated. The aqueous phase was extracted with ethyl acetate (3.times.100 ml). The. . .

DETD **Ammonium** acetate (10.6 g, 138 mmol) was evaporated from dry ethanol (100 mL), and redissolved in dry methanol (100 mL) over. . .

DETD . . . HPLC purification using a 25.times.200 mm C18 column and using a linear gradient of 25-40% acetonitrile in water containing 0.1M **ammonium** sulfate (pH 2.5). The product was purified in three runs and after ion exchange on a Waters Seppak C18 the. . .

DETD A solution of (2R)-2-(N-tert-butoxycarbonyl-N-methylamino)-3-phenylpropionic acid (4.00 g, 14.32 mmol) in N,N-dimethylformamide (10 ml) was cooled to 0.degree. C. **Ammonium** hydrogen carbonate (5.66 g, 71.60 mmol) was added as a solid. 1-Hydroxybenzotriazole hydrate (1.94 g, 14.32 mmol) and successively N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide. . .

DETD . . . completed, the solution was heated to reflux for 16 h. It was cooled to 5.degree. C. A 10% solution of **ammonium chloride** in water (60 ml) was added dropwise. The solution was warmed to 50.degree. C. for 1 h. It was cooled. . .

```
=> s ammonium chloride
L10      83717 AMMONIUM CHLORIDE

=> s catabolic
L11      31662 CATABOLIC

=> s muscle or protein
   3 FILES SEARCHED...
L12    7111621 MUSCLE OR PROTEIN

=> s l10 and l11 and l12
L13        160 L10 AND L11 AND L12

=> s l13 and py<2001
   3 FILES SEARCHED...
   4 FILES SEARCHED...
L14        114 L13 AND PY<2001

=> dup rem l14
PROCESSING COMPLETED FOR L14
L15         109 DUP REM L14 (5 DUPLICATES REMOVED)

=> d l15 1-109 ab bib kwic

L15  ANSWER 1 OF 109  USPATFULL
AB    A method for synthesizing C-glycosides of ulosonic acids such as
Neu5Ac,
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by which diastereocontrolled synthesis of .alpha.-C-glycosides of ulosonic acids is attained is disclosed. In the method of the present invention, an ulosonic acid sulfone or phosphite is reacted with an aldehyde or ketone compound in the presence of a lanthanide metal halide.

AN 2002:88629 USPATFULL
TI Method for synthesizing C-glycosides of ulosonic acids
IN Linhardt, Robert J., Iowa City, IA, United States
Vlahov, Iontcho R., Newark, DE, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 6376662 B1 20020423
WO 9831696 19980723 <--
AI US 1998-142937 19981117 (9)
WO 1998-JP129 19980116
19981117 PCT 371 date
PRAI US 1997-34953P 19970117 (60)
US 1997-35969P 19970121 (60)
US 1997-35986P 19970123 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Woodward, Michael P; Assistant Examiner: Moran, Marjorie A.
LREP Birch, Stewart, Kolasch & Birch, LLP
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 649
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6376662 B1 20020423
WO 9831696 19980723 <--
SUMM . . . oligosaccharides and suppressing undesired immune reactions (antirecognition phenomena); influencing the cell membrane permeability for permeability for ions, amino acids and **proteins**; and protection of glycoproteins against proteolysis.sup.1. Terminal Neu5Ac is an attachment site of pathogens to the cells and often **catabolic** and inflammatory processes are initiated on the removal of this carbohydrate group.sup.2. In general the "right" life time of a. . .
DETD . . . added dropwise at 20.degree. C. Stirring was continued for 45 min, than the reaction mixture was poured into an aqueous **ammonium chloride** solution and extracted twice with ethyl acetate. The combined organic layers were dried (MgSO.sub.4) and concentrated in vacuo. The residue. . .
DETD . . . added dropwise at 20.degree. C. Stirring was continued for 45 min, than the reaction mixture was poured into an aqueous **ammonium chloride** solution and extracted twice with ethyl acetate. The combined organic layers were dried (MgSO.sub.4) and concentrated in vacuo. The residue. . .
DETD . . . added dropwise at 20.degree. C. Stirring was continued for 45 min, than the reaction mixture was poured into an aqueous **ammonium chloride** solution and extracted twice with ethyl acetate. The combined organic layers were dried (MgSO.sub.4) and concentrated in vacuo. The residue. . .
DETD (3) Air, G. M.; Laver, W. G. **Proteins**: Structure, Function and Genetics, 1989, 6, 341-356, and references therein.

L15 ANSWER 2 OF 109 USPATFULL
AB The present invention is directed to methods for the modulation of cardiac function which comprise the administration of certain compounds,

as defined herein, having growth hormone secretagogue activity.

AN   2001:226599 USPATFULL
TI   Treatment of congestive heart failure with growth hormone secretagogues
IN   Kauffman, Raymond F., Carmel, IN, United States
        Palkowitz, Alan D., Carmel, IN, United States
PA   Eli Lilly and Company, Indianapolis, IN, United States (U.S. corporation)
PI   US 6329342   B1   20011211
        WO 9908697 19990225   <--
AI   US 2000-485924   20000218 (9)
        WO 1998-US17201   19980819
                                 20000218 PCT 371 date
                                 20000218 PCT 102(e) date
PRAI   US 1997-56135P   19970819 (60)
DT   Utility
FS   GRANTED
EXNAM   Primary Examiner: Henley, III, Raymond
LREP   Boudreaux, William R., Strode, Janelle D., McNeil, Scott A.
CLMN   Number of Claims: 33
ECL   Exemplary Claim: 1
DRWN   10 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 14373
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 6329342   B1   20011211
        WO 9908697 19990225   <--

SUMM   . . . forms of heart disease. Common causes of congestive heart failure include: narrowing of the arteries supplying blood to the heart **muscle** (coronary heart disease); prior heart attack (myocardial infarction) resulting in scar tissue large enough to interfere with normal function of. . . pressure; heart valve disease due to past rheumatic fever or an abnormality present at birth; primary disease of the heart **muscle** itself (cardiomyopathy); defects in the heart present at birth (congenital heart disease) and infection of the heart valves and/or **muscle** itself (endocarditis and/or myocarditis). Each of these disease processes can lead to congestive heart failure by reducing the strength of the heart **muscle** contraction, by limiting the ability of the heart's pumping chambers to fill with blood due to mechanical problems or impaired. . .

SUMM   . . . hormone is known to have the following basic effects on the metabolic processes of the body: (1) increased rate of **protein** synthesis in all cells of the body; (2) decreased rate of carbohydrate utilization in cells of the body; and (3). . .

SUMM   . . . also known as dwarfism. A deficiency in growth hormone secretion later in life may be characterized by excessive adiposity, reduced **muscle** mass, impaired exercise capacity, reduced body water, decreased bone mineral density, and psychological disorders. For example, a deficiency in growth. . .

DETD   . . . another composition which exhibits another activity, for example an antibiotic growth promoting agent, or a corticosteroid employed to minimize the **catabolic** side effects, or another compound which enhances efficacy and minimizes side effects. Growth promoting and anabolic agents include TRH, diethylstilbesterol,. . .

DETD   . . . dichloromethane (500 mL) at 0.degree. C. was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (12.3 g, 71.9 mmol).
After
18 h, ethyl acetate and saturated **ammonium chloride** were added and the mixture extracted with **ammonium chloride**, sodium bicarbonate, and brine. The organic extracts were dried over sodium sulfate and concentated. Purification by silica

gel chromatography (25%. . .
DETD . . . and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (1.36 g,
7.93
mmol). After 18 hours, ethyl acetate was added and the mixture washed with saturated aqueous **ammonium chloride**, saturated aqueous sodium bicarbonate, and brine. The organic extract was dried over sodium sulfate and concentrated. Purification by silica gel. . .
DETD . . . dichloromethane (500 mL) at 0 .degree. C. was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (12.3 g, 71.9 mmol).
After
18 h, ethyl acetate and **ammonium chloride** (saturated aqueous solution) were added and the resulting mixture extracted with aqueous **ammonium chloride**, aqueous sodium bicarbonate, and brine. The organic extracts were dried over sodium sulfate and concentrated. Purification by flash chromatography (25%. . .
DETD . . . dichloromethane (500 mL) at 0.degree. C. was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (12.3 g, 71.9 mmol).
After
18 h, ethyl acetate and saturated **ammonium chloride** were added and the mixture extracted with **ammonium chloride**, sodium bicarbonate, and brine. The organic extracts were dried over sodium sulfate and concentrated. Purification by silica gel chromatography (25%. . .

L15 ANSWER 3 OF 109 USPATFULL

AB This invention relates to the identification of homologs of atrazine chlorohydrolase and the use of these homologs to degrade s-triazine-containing compounds. In particular, this invention includes the identification of homologs of atrazine chlorohydrolase encoded by a DNA fragment having at least 95% homology to the sequence from the nucleic acid sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1, where the DNA fragment is capable of hybridizing under stringent conditions to SEQ ID NO:1 and has altered catalytic activity as compared with wild-type atrazine chlorohydrolase.

AN 2001:116807 USPATFULL
TI DNA molecules and **protein** displaying improved triazine compound degrading ability
IN Wackett, Lawrence P., St. Paul, MN, United States
Sadowsky, Michael J., Roseville, MN, United States
de Souza, Mervyn L., St. Paul, MN, United States
Minshull, Jeremy S., Menlo Park, CA, United States
PA Regents of the University of Minnesota, Minneapolis, MN, United States (U.S. corporation)
Maxygen Inc., Redwood City, CA, United States (U.S. corporation)
PI US 6265201 B1 20010724
WO 9831816 19980723
AI US 1998-155036 19980917 (9)
WO 1998-US944 19980116
19980917 PCT 371 date
19980917 PCT 102(e) date
PRAI US 1997-35404P 19970117 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Prouty, Rebecca E.; Assistant Examiner: Hutson, Richard
LREP Mueting, Raasch & Gebhardt, P.A.
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 16 Drawing Page(s)

LN.CNT 1381
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI DNA molecules and **protein** displaying improved triazine compound degrading ability
PI US 6265201 B1 20010724
WO 9831816 19980723
SUMM . . . isolated from a Pseudomonas sp. strain. See, for example, de Souza et al., Appl. Environ. Microbiol., 61, 3373 (1995). The **protein** expressed by the gene disclosed by de Souza et al., degrades atrazine, for example, at a V.sub.max of about 2.6 .mu.mol of hydroxyatrazine per min per mg **protein**. Although this is significant, it is desirable to obtain genes and the **proteins** they express that are able to dechlorinate triazine-containing compounds with chlorine moieties at an even higher rate and/or under a. . . in which the wild type enzyme is not stable, efficient, or active. Similarly, it is desirable to obtain genes and **proteins** encoded by these genes that degrade triazine-containing compounds such as those triazine containing compounds available under the trade names; "AMETRYN", "PROMETRYN", "CYANAZINE", "MELAMINE", "SIMAZINE", as well as TERBUTHYLAZINE and desethyldesisopiopylatriazine. It is also desirable to identify **proteins** expressed in organisms that degrade triazine-containing compounds in the presence of other nitrogen sources such as ammonia and nitrate.
SUMM . . . salt concentration, pH, improved activity in a soil environment, and the like, as compared with the wild-type atrazine chlorohydrolase (AtzA) **protein**.
SUMM The invention also relates to s-triazine-degrading **proteins** hlaving at least one amino acid different from the **protein** of SEQ ID NO:2, wherein the coding region of the nucleic acid encoding the s-triazine degrading **protein** has at least 95% homology to SEQ ID NO:1 and wherein the s-triazine-degrading **protein** has an altered catalytic activity as compared with the **protein** having the sequence of SEQ ID NO:2. In one embodiment, the **protein** is selected from the group consisting of SEQ ID NOS:5, 6 and 22-26. In one embodiment the substrate for the s-triazine degrading **protein** is ATRAZINE. In another embodiment the substrate for the s-triazine degrading **protein** is TERBUTHYLAZINE and in yet another embodiment the substrate for the s-triazine degrading **protein** is MELAMINE. In another embodiment this invention relates to a remediation composition comprising a cell producing at least one s-triazine-degrading **protein** having at least one amino acid different from the **protein** of SEQ ID NO:2, wherein the coding region of the nucleic acid encoding the s-triazine degrading **protein** has at least 95% homology to SEQ ID NO:1 and wherein the s-triazine-degrading **protein** has an altered catalytic activity as compared with the **protein** having the sequence of SEQ ID NO:2. In a preferred embodiment the composition is suitable for treating soil or water. In another embodiment the remediation composition comprises at least one s-triazine-degrading **protein** having at least one amino acid different from the **protein** of SEQ ID NO:2, wherein the coding region of the nucleic acid encoding the s-triazine degrading **protein** has at least 95% homology to SEQ ID NO:1 and wherein the s-triazine-degrading **protein** has an altered catalytic activity as compared with the **protein** having the sequence of SEQ ID NO:2. In a preferred embodiment this composition is also suitable for treating soil or water. In one embodiment the remediation composition comprises the **protein** bound to an immobilization support. In yet another embodiment, these

proteins are homotetramers, such as the homotetramers formed by AtzA.

SUMM In another embodiment the invention relates to a **protein** selected from the group consisting of **proteins** comprising the amino acid sequences of SEQ ID NOS:5, 6 and 22-26.

SUMM . . . hybridizing under stringent conditions to SEQ ID NO:1 and wherein there is at least one amino acid change in the **protein** encoded by the DNA fragment as compared with SEQ ID NO:2 and wherein the **protein** encoded by the DNA fragment is capable of dechlorinating at least one s-triazine-containing compound and has a catalytic activity different from the enzymatic activity of the **protein** of SEQ ID NO:2. In one embodiment the s-triazine-containing compound is ATRAZINE, TERBUTHYLAZINE, or MELAMINE. In one embodiment.

SUMM . . . to a method for treating a sample comprising an s-triazine containing compound comprising the step of adding a adding a **protein** to a sample comprising an s-triazine-containing compound wherein the **protein** is encoded by gene having at least a portion of the nucleic acid sequence of the gene having at least. . . of hybridizing under stringent conditions to SEQ ID NO:1, wherein there is at least one amino acid change in the **protein** encoded by the DNA fragment as compared with SEQ ID NO:2 and wherein the **protein** has an altered catalytic activity as compared to the **protein** having the amino acid sequence of SEQ ID NO:2. In one embodiment, the composition comprises bacteria expressing the **protein**. In one embodiment the s-triazine-containing compound is atrazine, in another the s-triazine-containing compound is TERBUTHYLAZINE and in another the s-triazine containing compound is (2,4,6-triamino-s-triazine). In one embodiment, the **protein** encoded by the gene is selected from the group consisting of SEQ ID NOS:5, 6 and 22-26.

SUMM . . . acid sequence encoding atrazine chlorohydrolase, mutagenizing the nucleic acid to obtain a modified nucleic acid sequence that encodes for a **protein** having an amino acid sequence with at least one amino acid change relative to the amino acid sequence of the atrazine chlorohydrolase, screening the **proteins** encoded by the modified nucleic acid sequence; and selecting **proteins** with altered catalytic activity as compared to the catalytic activity of the atrazine chlorohydrolase. Preferably, the atrazine chlorohydrolase nucleic acid. . .

DETD The present invention provides isolated and purified DNA molecules, and isolated and purified **proteins**, involved in the degradation of s-triazine-containing compounds. The **proteins** encoded by the genes of this invention are involved in the dechlorination and/or the deamination of s-triazine-containing, compounds. The wild type AtzA **protein** can catalyze the dechlorinationi of s-triazine-containing compounds but not the deamination of these compounds. The dechlorination reaction occurs on s-triazine. . .

DETD . . . of the genome of Pseudomonas sp. ADP (ADP is strain designiation for Atrazine-degrading Pseudomonas) bacterium. Specifically, these genomic fragments encode **proteins** involved in s-triazine dechlorination. The rate of degradation of atrazine that results from the expression of these fragments in E.. . . native Pseudomonas sp. strain ADP, this degradation in E. coli is unaffected by the presence of inorganic nitrogen sources like **ammonium chloride**. This is particularly advantageous for regions contaminated with nitrogen-containing fertilizers or herbicides, for

example. The expression of atrazine degradation activity. . .

DETD As used herein, the gene encoding a **protein** capable of dechlorinating atrazine and originally identified in Pseudomonas sp. strain ADP and expressed in E. coli is referred to as "atzA", whereas the **protein** that it encodes is referred to as "AtzA." Examples of the cloned wild type gene sequence and the amino acid. . . are provided as SEQ ID NO:1 and SEQ ID NO:2 respectively. As also used herein, the terms atrazine chlorohydrolase (AtzA) **protein**, atrazine chlorohydrolase enzyme, or simply atrazine chlorohydrolase, are used interchangeably, and refer to an atrazine chlorohydrolase enzyme involved in the. . .

DETD A "homolog" of atrazine chlorohydrolase is an enzyme derived from the gene sequence encoding atrazine chlorohydrolase where the **protein** sequence encoded by the gene is modified by amino acid deletion, addition, substitution, or truncation but that nonetheless is capable. . . chlorohydrolase (AtzA) has a nucleic acid sequence that is different from the atzA sequence (SEQ ID NO:1) and produces a **protein** with modified biological properties or, as used herein, "altered enzymatic activities." These homologs include those with altered catalytic rates as. . . salt concentration, pH, improved activity in a soil environment. and the like, as compared with the wild-type atrazine chlorolhydrolase (AtzA) **protein**. Thus, provided that two molecules possess enzymatic activity to an s-triazine-containing substrate and one molecule has the gene sequence of. . . of the homolog differs from SEQ ID NO:1 such that there is at least one amino acid change in the **protein** encoded by SEQ ID NO:1 (i.e., SEQ ID NO:2); 2) the homolog has different enzymatic characteristics from the **protein** encoded by SEQ ID NO:1 such as, but not limited to, an altered substrate preference, altered rate of activity, or. . . or the like, as discussed supra; and 3) where the coding region of the nucleic acid sequence encoding the variant **protein** has at least 95% homology to SEQ ID NO:1.

DETD As used herein, the terms "isolated and purified" refer to the isolation of a DNA molecule or **protein** from its natural cellular environment, and from association with other coding regions of the bacterial genome, so that it can. . .

DETD . . . i.e. recently evolved gene. That the gene is recently evolved is supported by the attributes of the gene and the **protein** encoded by the gene. For example: (i) the gene has a limited s-triazine range that includes atrazine and the structurally. . . act on all s-triazines; (ii) the gene has a high sequence homology to genes isolated from other bacteria that produce **proteins** with atrazine-degrading activity; (iii) is not organized with the atzB and atzC genes in a contiguous arrangement such as an. . .

DETD . . . from a survey of atrazine-degrading bacteria are so structurally and catalytically similar suggest that the atza gene and the AtzA **protein** can be improved and will be improved naturally over time. Indeed, most biodegradative enzymes are orders of magnitude below diffusion limiting enzyme rates and, under this hypothesis, are also candidates for gene and **protein** modifications.

DETD . . . embodiment of this invention, a method is disclosed for selecting or screening modified and improved atzA gene sequences that encode **protein** with improved enzymatic activity, whether the activity is enzymatic rate, using atrazine as a substrate, as compared to the wild-type. . . to obtain altered atzA sequences, selecting or

screening for clones expressing altered AtzA activity and selecting gene
sequences encoding AtzA **protein** with improved s-triazine-degrading activity.

DETD There are a number of methods in use for creating mutant **proteins** in a library format from a parent sequence. These include the polymerase chain reaction (Leung, D. W. et al. Technique.

.

. et al., Gene 44:177-183 (1986), Hermes, J. D. et al., Proc. Natl. Acad. Sci. USA 87:696-700 (1990), Delgrave et al. **Protein** Engineerinn 6:327-331, (1993), Delgrave et al. Bio/Technology 1:1548-1552 (1993), and Goldman, E R et al., Bio/Technology 10: 1557-1561 (1992)), as. . .

DETD Once intact gene sequences are reassembled, they are incorporated into a
vector suitable for expressing **protein** encoded by the reassembled nucleic acid, or as provided in Example 1, where the gene sequences are already in a. . . The host, generally an E. coli species, is used in assays to screen or select for clones expressing the
AtzA **protein** under defined conditions. The type of organism can be matched to the mutagenesis procedure and in Example 2, a preferred. . .

DETD . . . assays suitable for use in this invention can take any of a variety of forms for determining whether a particular **protein** produced by the organism containing the variant atzA sequences expresses
an enzyme capable of dechlorinating or deaminating s-triazine compounds.
Therefore,. . .

DETD . . . can be altered to a pH range of about 5 to about 9. These assays would likely use isolated homolog **protein** to permit an accurate assessment of the effect of pH. The assay, or a modification of
the assay, suitable for. . .

DETD . . . rule out if the apparent enhanced activity of the enzyme is the
result of a faster or more efficient AtzA **protein** production or whether the effect observed is the result of an altered atzA gene sequence. For example, in Example 2,. . .

DETD . . . homologs are isolated for further analysis. Clones containing putative faster enzyme(s) can be picked, grown in liquid culture, and the **protein** homolog can be purified, for example, as described (de Souza et al. J. Bacteriology, 178:4894-4900 (1996)). The genes encoding the. . . as known in the art, for extracellular expression or the homologs can be purified from bacteria. An exemplary method for **protein** purification is provided in Example 4. In a preferred method, **protein** was collected from bacteria using ammonium sulfate precipitation and further purified by HPLC (see for example, de Souza et al.,. . .

DETD . . . found to have at least a 10 fold higher activity and contained 8 different amino acids than the native AtzA **protein** (A7 and T7, see FIGS. 1-4). A subsequent round of DNA shuffling starting with the homolog gene sequence yielded further. . . enzyme and other AtzA homologs (clones A40, A42, A44, A46, A60 corresponding to nucleic acid SEQ ID NOS:17-21 and to **protein** SEQ ID NOS:22-26, respectively) represent **catabolic** enzymes modified in their biological activity. Preferred homologs identified in initial studies include A7, T7, A11, A44, and A46.

DETD . . . and the kinetic improvement of the homologs has important

implications for enzymatic environmental remediation of this widely used herbicide. Less **protein** is required to dechlorinate the same amount of atrazine. Importantly, the **protein** can also be used for degradation of the s-triazine-compound TERBUTHYLAZINE.

DETD This invention also relates to nucleic acid and **protein** sequences identified from the homologs of this invention. Peptide and nucleic acid fragments of these sequences are also contemplated and. . . . of this invention. The homologs of this invention include those with an activity different from the native atrazine chlorohydrolase (AtzA) **protein**. As noted stipra. an activity that is different from the native atrazine chlorohydrolase **protein** includes enzymatic activity that is improved or is capable of functioning under different conditions such as salt concentration, temperature, altered. . . the homologs hybridize to a DNA molecule complementary to the wild-type coding region of a DNA molecule encoding wild-type AtzA **protein** , such as the sequence provided in SEQ ID NO:1, under high to moderate stringency hybridization conditions. The homologs preferably have. . .

DETD . . . ID NO:4. FIG. 3 provides the amino acid sequence alignment of SEQ ID NO:2, the amino acid sequence of the **protein** encoded by SEQ ID NO:1, with SEQ ID NO:5 and FIG. 4 provides the amino acid sequence alignment of SEQ. . .

DETD . . . this invention, the success attributed to the identification of homologs of AtzA may be based on the recognition that this **protein** is not evolutionarily mature. Therefore, not all gene sequences are good candidates as the starting material for identifying a number of biological variants of a particular **protein** and similarly, not all enzymes are amenable to the order of magnitude of rate enhancement by directed evolution using DNA. . .

DETD . . . selected for expression, and will be apparent to those skilled in the art. Induction of cells to express the AtzA **protein** is accomplished using the procedures required by the particular expression system selected. The host cells referred to in this disclosure. . .

DETD This invention also relates to isolated **proteins** that are the product of the gene sequences of this invention. The isolated **proteins** are **protein** homologs of the wild-type atrazine chlorohydrolase enzyme despite their potential for altered substrate preference. The **protein** can be isolated in a variety of methods disclosed in the art and a preferred method for isolating the **protein** is provided in Examples 4 and 5 and in the publications of de Souza et al. (supra).

DETD The wild-type AtzA **protein** acts on Atrazine, desethylatrazine, Desisopropylatrazine and SIMAZINE but did not degrade Desethyldesisopropylatrazine or MELAMINE and only poorly degraded TERBUTHYLAZINE. Homologs identified in this invention have a spectrum of substrate preferences identical to the wild-type AtzA **protein** and in addition, for example, are able to degrade other substrates such as TERBUTHYLAZINE. That homologs were identified that were. . . used on the wild-type progenitor atzA gene or on the homologs produced by this invention to produce even more useful **proteins** for environmental remediation of s-triazine-containing compounds. Example 7 provides an assay for detecting degradation, including deamination, of a soluble s-triazine-containing. . .

DETD Various environmental remediation techniques are known that utilize high

levels of **proteins**. Bacteria or other hosts expressing the homologs of this invention can be added to a remediation mix or mixture in need of remediation to promote contaminate degradation. Alternatively, isolated AtzA homologs can be added. **Proteins** can be bound to immobilization supports, such as beads, particles, films, etc., made from latex, polymers, alginate, polyurethane, plastic, glass, polystyrene, and other natural and man-made support materials. Such immobilized **protein** can be used in packed-bed columns for treating water effluents. The **protein** can be used to remediate liquid samples, such as contaminated water, or solids. The advantage of some of the homologs. . .

DETD . . . DNA fragments. The GCG sequence analysis software package (Genetics Computer Group, Inc., Madison, Wis.) was used for all DNA and **protein** sequence comparisons. Radiolabelled chemicals were obtained from Ciba Geigy Corp., Greensboro, N.C.

DETD **Protein** Purification of AtzA or Homologs

DETD . . . type atzA gene or alternatively with a homolog, in a vector capable of directing expression of the gene as a **protein**, was grown overnight at 37.degree. C. in eight liters of LB medium containing 25 .mu.g/ml chloramphenicol. The culture medium was. . .

DETD Where purified **protein** was desired, the solution was loaded onto a Mono Q HR 16/10 Column (Pharmacia LKB Biotechnology, Uppsala, Sweden). The column was washed with 25 mM MOPS buffer (pH 6.9), and the **protein** was eluted with a 0-0.5 M KCl gradient. **Protein** eluting from the column was monitored at 280 nm by using a Pharmacia U.V. **protein** detector. Pooled fractions containing the major peak were dialyzed overnight against 1 liter 25 mM MOPS buffer (pH 6.9). The. . .

DETD **Protein** Verification: **Protein** subunit sizes were determined by SDS polyacrylamide gel electrophoresis by comparison to known standard **proteins**, using a Mini-Protean II gel apparatus (Biorad, Hercules, Calif.). The size of the holoenzyme was determined by gel filtration chromatography on a Superose 6 HR (1.0.times.30.0 cm) column, using an FPLC System (Pharmacia, Uppsala, Sweden). The **protein** was eluted with 25 mM MOPS buffer (pH 6.9) containing 0.1 M NaCl. **Proteins** with known molecular weights were used as chromatography standards. Isoelectric point determinations were done using a Pharmacia Phast-Gel System and. . .

DETD Enzyme Kinetics. Purified AtzA **protein** and homologs of the **protein** at 50 .mu.g/ml, were separately added to 500 .mu.l of different concentrations of atrazine (23.3 .mu.M, 43.0 .mu.M, 93 .mu.M,. . .

DETD . . . degradation. From experiments done with Pseudomonas species strain ADP on solid media with 500 ppm atrazine and varying concentrations of **ammonium chloride**, **ammonium chloride** concentrations as low as 0.6-1.2 mM were sufficient to inhibit visible clearing on the plates, even after 2 weeks of. . . using E. coli DH5.alpha. (pMD1 or pMD2) and other E. coli strains, atrazine degradation was observed in the presence of **ammonium chloride** concentrations as high as 48 mM. This value is almost 40-80 fold higher than the wild-type tolerance for **ammonium chloride** with concomitant atrazine degradation. Therefore, it was not necessary to use media free of exogenous ammonia in the screening assays.

DETD . . . of TERBUTHYLAZINE degradation. Sample 1 is a control sample

without enzyme. Sample 2 uses a two fold excess of AtzA **protein**
as compared to the concentration of homolog added in Sample 3 and
Sample
4. Sample 3 employed the T7 homolog. . . .

DETD . . . Example 2 are subjected to further mutagenesis and colonies capable of growing in MELAMINE can be identified. Colonies containing the **protein** AtzA are tested for growth in MELAMINE under identical conditions. Other s-triazine containing compounds such as the pesticides available under. . .

L15 ANSWER 4 OF 109 USPATFULL

AB This invention is directed to compounds of the formula ##STR1##

and the pharmaceutically-acceptable salts thereof, where the substituents are as defined in the Specification, which are growth hormone secretogogues and which increase the level of endogenous growth hormone. The compounds of this invention are useful for the treatment and prevention of osteoporosis and/or frailty, congestive heart failure, frailty associated with aging, obesity; accelerating bone fracture repair, attenuating **protein catabolic** response after a major operation, reducing cachexia and **protein** loss due to chronic illness, accelerating wound healing, or accelerating the recovery of burn patients or patients having undergone major surgery; improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis. The compounds of the present invention are also useful in treating osteoporosis and/or frailty when used in combination with: a bisphosphonate compound such as alendronate; estrogen, premarin, and optionally progesterone; an estrogen agonist or antagonist; or calcitonin, and pharmaceutical compositions useful therefor. Further, the present invention is directed to pharmaceutical compositions useful for increasing the endogenous production or release of growth hormone in a human or other animal which comprises an effective amount of a compound of the present invention and a growth hormone secretagogue selected from GHRP-6, Hexarelin, GHRP-1, growth hormone releasing factor (GRF), IGF-1, IGF-2 or B-HT920. The invention is also directed to intermediates useful in the preparation of compounds of Formula I.

AN 2001:97924 USPATFULL
TI Dipeptide derivatives as growth hormone secretagogues
IN Carpino, Philip Albert, Groton, CT, United States
Griffith, David Andrew, Old Saybrook, CT, United States
Lefker, Bruce Allen, Gales Ferry, CT, United States
PA Pfizer Inc., New York, NY, United States (U.S. corporation)
PI US 6251902 B1 20010626
WO 9858947 19981230 <--
AI US 1999-380887 19990908 (9)
WO 1998-IB873 19980605
19990908 PCT 371 date
19990908 PCT 102(e) date
PRAI US 1997-50764P 19970625 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Raymond, Richard L.
LREP Richardson, Peter C., Benson, Gregg C., Ronau, Robert T.

CLMN Number of Claims: 50
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 6506
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6251902 B1 20010626
WO 9858947 19981230 <--

AB . . . treatment and prevention of osteoporosis and/or frailty, congestive heart failure, frailty associated with aging, obesity; accelerating bone fracture repair, attenuating **protein catabolic** response after a major operation, reducing cachexia and **protein** loss due to chronic illness, accelerating wound healing, or accelerating the recovery of burn patients or patients having undergone major surgery; improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis. The compounds of the present invention are also useful. . .

SUMM 1. Increased rate of **protein** synthesis in substantially all cells of the body;

SUMM . . . in lean body mass and concomitant increase in total body fat, particularly in the truncal region. Decreased skeletal and cardiac **muscle** mass and **muscle** strength lead to a significant reduction in exercise capacity. Bone density is also reduced. Administration of exogenous growth hormone has. . .

SUMM . . . D., et al, Horm Res 36 (Suppl 1):73 (1991)) has been shown to produce increases in lean body, hepatic and **muscle** mass while decreasing fat mass. Thus, GH therapy for obesity would seem attractive except for the diabetogenic effects of GH.

SUMM methods for accelerating bone fracture repair, attenuating **protein catabolic** response after a major operation, reducing cachexia and **protein** loss due to chronic illness such as AIDS or cancer, accelerating would healing, or accelerating the recovery of burn patients. . .

SUMM methods for improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis, which methods comprise administering to a human or other. . .

SUMM methods for increasing piglet number, increasing pregnancy rate in sows, increasing viability of piglets, increasing weight of piglets or increasing **muscle** fiber size in piglets which comprise administering to a sow or piglet an effective amount of a compound of Formula. . .

SUMM methods for increasing **muscle** mass, which comprise administering to a human or other animal such as dogs, cats, horses, cattle, pigs, chickens, turkeys, sheep. . .

SUMM In yet another aspect, this invention provides methods for improving **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis and renal homeostasis, which comprise administering to a human or other animal. . .

SUMM . . . arylacetyl and .alpha.-aminoacyl, or .alpha.-aminoacyl-.alpha.-aminoacyl wherein said .alpha.-aminoacyl moieties are independently any of the naturally occurring L-amino acids found in **proteins**,

SUMM . . . cats, camels and horses; treating growth hormone deficient adult humans or other animals especially dogs, cats, camels and horses; preventing **catabolic** side effects of glucocorticoids, treating osteoporosis, stimulating the immune system, accelerating wound healing, accelerating bone fracture repair, treating growth retardation,. . . osteochondrodysplasias, Noonans syndrome, sleep disorders, Alzheimer's

disease, delayed wound healing, and psychosocial deprivation; treating pulmonary dysfunction and ventilator dependency; attenuating **protein catabolic** response after a major operation; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treating hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to prevent. . . of thymic function; adjunctive therapy for patients on chronic hemodialysis; treating immunosuppressed patients and enhancing antibody response following vaccination; improving **muscle** strength, increasing **muscle** mass, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulating osteoblasts, bone remodeling, and cartilage. . .

SUMM . . . stimulation of pre- and post- natal growth, enhanced feed efficiency in animals raised for meat production, improved carcass quality (increased **muscle** to fat ratio) (Campbell, R. G. et al., (1989), J. Anim. Sci. 67, 1265; Dave, D. J., Bane, D. P.,. . . antibody response following vaccination or improved developmental processes; and may have utility in aquaculture to accelerate growth and improve the **protein**-to-fat ratio in fish.

SUMM . . . 14-22; Mankin. J. J. et al., J. of Bone and Joint Surgery, Vol. 60-A, #8, Dec. 1978, pp. 1071-1075); attenuating **protein catabolic** response after major surgery, accelerating recovery from burn injuries and major surgeries such as gastrointestinal surgery; stimulating the immune system. . . heart failure, treating acute or chronic renal failure or insufficiency, treating obesity; treating growth retardation, skeletal dysplasia and osteochondrodysplasias; preventing **catabolic** side effects of glucocorticoids; treating Cushing's syndrome; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer; accelerating weight gain and **protein** accretion in animals receiving total parenteral nutrition; providing adjuvant treatment for ovulation induction and to prevent gastrointestinal ulcers; improving **muscle** mass, strength and mobility; maintenance of skin thickness, and improving vital organic function and metabolic homeostasis.

SUMM . . . hereby incorporated by reference. In another aspect, this invention provides methods for accelerating bone fracture repair and wound healing, attenuating **protein catabolic** response after a major operation, and reducing cachexia and **protein** loss due to chronic illness, which comprise administering to a human or another animal, especially dogs, cats and horses in. . .

DETD . . . reaction was quenched with methanol and concentrated in vacuo. Ethyl acetate was added, and the mixture was extracted with saturated **ammonium chloride** solution, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo to give crude product. Purification by silica gel. . .

DETD . . . temperature and stirred for about 2.5 hours. The reaction mixture was cooled to about 0.degree. C., and quenched with saturated **ammonium chloride** solution. The mixture was then diluted with ethyl acetate, and washed three times with saturated sodium bicarbonate solution, twice with. . .

DETD . . . 0.0222 mmol) portionwise. The reaction was stirred for about 3 hours at room temperature. The reaction was quenched with saturated **ammonium chloride** solution, the methanol was removed

in vacuo, and the aqueous mixture was extracted several times with
ethyl
acetate. The combined. . .

L15 ANSWER 5 OF 109 USPATFULL
AB Compounds of peptide mimetic nature having the general formula I ##STR1## wherein a and b are independently 1 or 2, R.sup.1 and R.sup.2 are independently H or C.sub.1-6 alkyl, G and J are independently,
inter
alia, aromats, and D and E are independently several different groups are growth hormone secretagogous with improved bioavailability.
AN 2000:131863 USPATFULL
TI Compounds with growth hormone releasing properties
IN Hansen, Thomas Kruse, Herlev, Denmark
Peschke, Bernd, Maaloev, Denmark
Lau, Jesper, Farum, Denmark
Lundt, Behrend Friedrich, Kokkedal, Denmark
Ankersen, Michael, Frederiksberg, Denmark
Watson, Brett, Vaerloese, Denmark
Madsen, Kjeld, Vaerloese, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 6127391 20001003 <--
AI US 1998-218686 19981221 (9)
RLI Division of Ser. No. US 1996-769020, filed on 18 Dec 1996
PRAI DK 1995-1462 19951222
DK 1996-698 19960625
DK 1996-812 19960724
DK 1996-1248 19961106
US 1996-22062P 19960722 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Aulakh, Charanjit S.
LREP Zelson, Steve T., Rozek, Carol E.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 8344
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6127391 20001003 <--
SUMM . . . of growing. In addition, growth hormone is known to have a number of effects on metabolic processes, e.g., stimulation of **protein** synthesis and free fatty acid mobilisation and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism.. . .
SUMM In disorders or conditions where increased levels of growth hormone is desired, the **protein** nature of growth hormone makes anything but parenteral administration non-viable. Furthermore, other directly acting natural secretagogues, e.g., GHRH and PACAP,. . .
SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids, prevention and treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating borte. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation, treatment of pulmonary dysfunction and ventilator dependency, attenuation of **protein** **catabolic** responses after major surgery, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis, adjuvant treatment for ovulation induction; to stimulate thymic development and

prevent the age-related decline of thymic function, treatment of immunosuppressed patients, improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal homeostasis in the frail elderly, stimulation of osteoblasts, bone remodelling and. . .

DETD . . . mixture was stirred for 1.5 h at -78.degree. C. and then warmed to room temperature. A 10% aqueous solution of **ammonium chloride** (200 ml) was added dropwise. The phases were separated. The aqueous phase was extracted with ethyl acetate (3.times.100 ml). The. . .

DETD . . . completed, the solution was heated to reflux for 16 h. It was cooled to 5.degree. C. A 10% solution of **ammonium chloride** in water (60 ml) was added dropwise. The solution was warmed to 50.degree. C. for 1 h. It was cooled. . .

L15 ANSWER 6 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 2000:125226 USPATFULL

TI Intermediates for preparation of steroid receptor modulator compounds

IN Jones, Todd K., Solana Beach, CA, United States
Winn, David T., San Diego, CA, United States
Hamann, Lawrence G., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States
Farmer, Luc J., La Jolla, CA, United States
Davis, Robert L., Santee, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)

PI US 6121450 20000919 <--

AI US 1997-947427 19971008 (8)

RLI Division of Ser. No. US 1995-462643, filed on 5 Jun 1995, now patented, Pat. No. US 5696130 which is a continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Huang, Evelyn Mei

LREP Elmer, J. Scott, Respess, William L.

CLMN Number of Claims: 2

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 10966

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6121450 20000919 <--

SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .

DETD . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate,

protein and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.

DETD . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrates, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.

DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .

DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .

DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .

DETD . . . partial agonists which mimnic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .

DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . for the transcription-modulating activity of the target IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g., [.sup.3. . .

DETD To date, binding assays have not been performed utilizing ER or MR

proteins.
DETD . . . concentration (nM), requivuired to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.
DETD The gain and loss of sexual organ weights reflect the changs of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J. Urol., 188-191 (1991), the disclosure of. . .

L15 ANSWER 7 OF 109 USPATFULL
AB The present invention is directed to certain compounds of the general structural formula: ##STR1## wherein R.sub.1, R.sub.1a, R.sub.2a, R.sub.3, R.sub.3a, R.sub.4, R.sub.5, R.sub.6, A, W, and n are as defined herein. These compounds promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, such as short stature in growth hormone deficient children, and to treat medical conditions which are improved by the anabolic effects of growth hormone.
Growth hormone releasing compositions containing such compounds as the active ingredient thereof are also disclosed.
AN 2000:125101 USPATFULL
TI Naphthyl compounds promote release of growth hormone
IN Chen, Meng Hsin, Westfield, NJ, United States
Morriello, Gregori J., Belleville, NJ, United States
Nargund, Ravi, East Brunswick, NJ, United States
Patchett, Arthur A., Westfield, NJ, United States
Yang, Lihu, Edison, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 6121325 20000919 <--
AI US 1997-826290 19970327 (8)
RLI Division of Ser. No. US 1995-398247, filed on 3 Mar 1995, now patented, Pat. No. US 5663171, issued on 2 Sep 1997 which is a continuation-in-part of Ser. No. WO 1994-US13596, filed on 23 Nov 1994 which is a continuation-in-part of Ser. No. US 1993-157774, filed on 24 Nov 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Ambrose, Michael G.
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2345
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6121325 20000919 <--
SUMM . . . hormone is known to have the following basic effects on the metabolic processes of the body: (1) Increased rate of **protein** synthesis in all cells of the body; (2) Decreased rate of carbohydrate utilization in cells of the body; (3) Increased. . .
SUMM . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active

materials wherein the combination enhances efficacy and minimizes side effects.

SUMM . . . uses as growth hormone itself. These varied uses may be summarized as follows: treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system, acceleration of wound healing; accelerating bone fracture repair;. . .

syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein** **catabolic** response after a major operation; treating malabsorption syndromes; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . virus; treatment of syndromes manifested by non-restorative sleep and musculoskeletal pain, including fibromyalgia syndrome or chronic fatigue syndrome; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .

SUMM . . . the instant compounds are useful in the prevention or treatment of a condition selected from the group consisting of: osteoporosis; **catabolic** illness; immune deficiency, including that in individuals with a depressed T.sub.4 /T.sub.8 cell ratio; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and **protein** loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or burns,. . .

DETD . . . TiCl.sub.4 (0.46 ml) was added. After stirring 2.5 hour at 0.degree. C., this clear solution was quenched with saturated aqueous **ammonium chloride**. This mixture was extracted with methylene chloride, washed with sodium bicarbonate, brine and dried over sodium sulfate. Concentration and purification. . .

CLM What is claimed is:
7. The method of claim 6 wherein the disease or condition is selected from the group consisting of: osteoporosis; **catabolic** illness; immune deficiency; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and **protein** loss due to chronic illness; and the treatment of patients recovering from major surgery, wounds or burns.

L15 ANSWER 8 OF 109 USPATFULL

AB Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

AN 2000:124800 USPATFULL

TI Altered polypeptides with increased half-life

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Snedecor, Bradley R., Portola Valley, CA, United States

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EXNAM   Primary Examiner: Saunders, David
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CLMN   Number of Claims: 23
ECL   Exemplary Claim: 1
DRWN   4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 3411
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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SUMM . . . (pFc') fragment of human IgG also produced by trypsin digestion of the Fc fragment was rapidly eliminated, indicating that the **catabolic** site of IgG is located in the CH2 domain. Ellerson et al., J. Immunol., 116: 510 (1976); Yasmeen et al.,. . .

SUMM The **catabolic** rates of IgG variants that do not bind the high-affinity Fc receptor FcRI or C1q are indistinguishable from the rate of clearance of the parent wild-type antibody, indicating that the **catabolic** site is distinct from the sites involved in FcRI or C1q binding. Wawrzynczak et al., Molec. Immunol., 29: 221 (1992).. . .

SUMM Staphylococcal **protein** A-lgG complexes were found to clear more rapidly from the serum than uncomplexed IgG molecules. Dima et al., Eur. J.. . . on the pharmacokinetics of the Fc-hinge fragment. The authors showed that the site of the IgG1 molecule that controls the **catabolic** rate (the "**catabolic** site") is located at the CH2-CH3 domain interface and overlaps with the Staphylococcal **protein** A binding site. See also WO 93/22332 published Nov. 11, 1993. The concentration catabolism phenomenon is also studied in Zuckier. . .

SUMM WO 94/04689 discloses a **protein** with a cytotoxic domain, a ligand-binding domain and a peptide linking these two domains comprising an IgG constant region domain having the property of increasing the half-life of the **protein** in mammalian serum.

SUMM A stereo drawing of a human Fc fragment and its complex with fragment B of **Protein** A from Staphylococcus aureus is provided by Deisenhofer, Biochemistry, 20: 2364 (1981).

DETD . . . as is well known to those skilled in the art of antibody technology. Examples of such polypeptides are peptides and **proteins**, whether from eukaryotic sources such as, e.g., yeast, avians, plants, insects, or mammals, or from bacterial sources such as, e.g.,. . .

DETD . . . hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as **Protein** C; atrial naturietic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA);. . . a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial **protein**, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; **protein** A or D; rheumatoid factors; a neurotrophic factor such as brain-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3,. . .

TGF-.beta.2, TGF.beta.3, TGF-.beta.4, or TGF-.beta.5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding **proteins**; CD **proteins** such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic **protein** (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs),. . . IL-1 to IL-10; an anti-HER-2
antibody
without a native Fc region of an IgG; superoxide dismutase; T-cell receptors; surface membrane **proteins**; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport **proteins**; homing receptors; addressins; regulatory **proteins**; antibodies without a native Fc region of an IgG; and fragments of any of the above-listed polypeptides.

DETD Libraries are screened with probes designed to identify the gene of interest or the **protein** encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to. . .

DETD . . . In some preferred embodiments, the nucleic acid sequence includes the polypeptide of interest's signal sequence. Nucleic acid having all the **protein** coding sequence is obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for. . .

DETD . . . insertions include insertions to the internal portion of the polypeptide of interest, as well as N- or C-terminal fusions with **proteins** or peptides containing the desired epitope that will result, upon fusion, in an increased half-life.

DETD . . . bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for **protein** production, generally an expression vector of the type typically employed for transformation of an appropriate host.

DETD Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a **protein** necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode **proteins** that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or. . .

DETD . . . drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a **protein** conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et. . .

DETD . . . that encodes the polypeptide variant. Amplification is the process by which genes in greater demand for the production of a **protein** critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the. . .

DETD . . . cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the polypeptide variant, wild-type DHFR **protein**, and another selectable marker such as aminoglycoside 3-phosphotransferase (APH) can be selected by cell growth in medium containing a selection. . .

DETD . . . amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding **proteins** endogenous to the host, with examples of such hosts including E. coli W3110 strain 1A2, which has the complete genotype. .

DETD . . . Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-**protein** duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound. . . .

DETD . . . step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration; optionally, the **protein** may be concentrated with a commercially available **protein** concentration filter, followed by separating the polypeptide variant from other impurities by one or more steps selected from immunoaffinity chromatography,. . . groups), chromatography on Blue-Sepharose, CM Blue-Sepharose, MONO-Q, MONO-S, lentil lectin-Sepharose, WGA-Sepharose, Con A-Sepharose, Ether Toyopearl, Butyl Toyopearl, Phenyl Toyopearl, or **protein** A Sepharose, SDS-PAGE chromatography, silica chromatography, chromatofocusing, reverse phase HPLC (e.g., silica gel with appended aliphatic groups), gel filtration using,. . .

DETD . . . another embodiment, supernatants from systems which secrete recombinant polypeptide variant into culture medium are first concentrated using a commercially available **protein** concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate may be applied to a suitable purification matrix. For example, a suitable affinity matrix may comprise a ligand for the **protein** , a lectin or antibody molecule bound to a suitable support. Alternatively, an anion-exchange resin may be employed, for example, a. . . matrix or substrate having pendant DEAE groups. Suitable matrices include acrylamide, agarose, dextran, cellulose, or other types commonly employed in **protein** purification. Alternatively, a cation-exchange step may be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups.. . .

DETD Mammalian polypeptide variant synthesized in recombinant culture is characterized by the presence of non-human cell components, including **proteins**, in amounts and of a character which depend on the purification steps taken to recover the polypeptide variant from culture.. . .

DETD . . . O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using .sup.125 I or .sup.131 I to prepare labeled **proteins** for use in radioimmunoassay, the chloramine T method described above being suitable.

DETD . . . of seryl or threonyl residues, methylation of the .alpha.-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, **Proteins**: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and. . .

DETD . . . the compound tunicamycin as described by Duskin et al., J. Biol. Chem., 257: 3105 (1982). Tunicamycin blocks the formation of **protein**-N-glycoside linkages.

DETD . . . be fused to a second polypeptide and the antibody or fusion thereof may be used to isolate and purify the **protein** to which it binds from a source such as a CD11 or CD18 antigen. In another embodiment, the invention provides. . .

DETD . . . as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; **proteins**, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine. . .

DETD . . . (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a **protein** that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin. . .

DETD . . . the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different **protein** and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as **protein** fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

DETD . . . as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the **protein** used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable. . .

DETD . . . are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, **protein** A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DETD . . . E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin **protein**, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of. . .

DETD Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic **protein** chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a. . .

DETD . . . using leucine zippers. Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun **proteins** were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the. . .

DETD . . . tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or variant antibody, and **proteins** that bind to the analyte are denominated binding partners, whether they be antibodies, cell-surface receptors, or antigens.

DETD . . . flanking the F1 origin were removed from pB0475 and DNA coding for anti-CD18 Fab H52, version OZ (Eigenbrot et al., **Proteins**, 18: 49-62 [1994]) was substituted for DNA coding for human growth hormone using the EcoRV and Sphl sites. Hence, pH52. . .

DETD . . . technical (Difco.TM.0231-01-0), 0.3 g yeast extract certified (Difco.TM.0127-01-7), 0.19 g MgSO.sub.4 anhydrous or 0.394 g MgSO.sub.4.7H.sub.2 O (Sigma.TM.M2773), 1.07 g **ammonium chloride** (Sigma.TM. A9434), 0.075 g KCl (Sigma.TM.P5405), 4.09 g NaCl (Sigma.TM. S3014), 120.0 mL of 1 M triethanolamine pH 7.4, qs. . .

DETD The supernatant was then passed over a **Protein** G-Sepharose.TM.

Fast Flow (Pharmacia) column [0.5 mL bed volume] previously
equilibrated
by passing 10 mL TE buffer through the column.. . . added to concentrated eluant, and the resulting mixture was re-concentrated to 0.5 mL. SDS-PAGE gels were run to ascertain that **protein** had been produced.

DETD . . . out on a reverse-phase PLRP-S.TM. 4.6.times.50 mm column, 8-mm particle size (Polymer Laboratories, Shropshire, UK), maintained at 50.degree. C. The **proteins** were eluted using an increasing linear gradient from 31% B to 41% B. Buffer A contained 0.1% trifluoroacetic acid in. . . and Buffer B contained 0.1% trifluoroacetic acid in HPLC-grade acetonitrile. The flow rate was maintained at 2 mL/min, and the **protein** profile was monitored at 214 nm.

DETD . . . carried out on a Bakerbond carboxy-sulfon (CSX).TM. 50.times.4.6 mm column (J. T. Baker Phillipsburg, N.J.), maintained at 55.degree. C. The **proteins** were eluted using an increasing linear gradient from pH 6.0 to pH 8.0 at a flow rate of 2 mL/min. . . .

DETD SDS-PAGE analysis was carried out on precast Novex.TM. gels (Novex, San Diego, Calif.). The **proteins** were stained using the Morrissey silver stain method. Morrissey, Anal. Biochem., 117: 307-310 (1981).

DETD . . . test (Associates of Cape Cod Inc., Woods Hole, Mass.). Samples containing less than 2 endotoxin units (Eu) per mg of **protein** were used in the pharmacokinetic studies.

DETD . . . 3.5. To this solution, pepsin (1 mg/mL) dissolved in 100 mM sodium citrate buffer, pH 3.5, was added at a pepsin-to-**protein** ratio of 1:12. After 4 hours at room temperature, the mixture's pH was raised to pH 6.4 with 10% NaOH.

DETD . . . described above for the Fab antibody fragment variants. After endotoxin determinations, samples containing less than 2 Eu per mg of **protein** were used in the pharmacokinetic studies set forth below.

L15 ANSWER 9 OF 109 USPATFULL

AB Disclosed are CHO cells which are capable of continued production of human LH-RH receptor **proteins**, or cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or partial peptides thereof; methods for screening compounds which have affinity for an LH-RH receptor by contacting the compound with the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the partial peptides thereof; kits for screening them; the compounds which have affinity for the LH-RH
receptor
obtained by methods for the screening or kits for the screening; and pharmaceutical compositions containing the compound, thereby being able to early provide prophylactic or therapeutic compositions, for example, for prostate cancer, uterine cancer, breast cancer, a pituitary tumor, endometriosis, hysteromyoma or precocious puberty. They are also useful as a pregnancy controlling composition such as contraceptive or a menstrual cycle controlling composition.

AN 2000:121288 USPATFULL

TI Human LH-RH receptor expression cells and use thereof

IN Onda, Haruo, Tsuchiura, Japan
Ohkubo, Shoichi, Tsukuba, Japan
Hinuma, Shuji, Tsukuba, Japan

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AI US 1997-867260 19970602 (8)

RLI Division of Ser. No. US 1995-423691, filed on 18 Apr 1995, now
patented,

Pat. No. US 5677184
PRAI JP 1994-80731 19940419
JP 1994-218349 19940913
DT Utility
FS Granted
EXNAM Primary Examiner: Caputa, Anthony C.; Assistant Examiner: Hayes, Robert C.
LREP Dike, Bronstein, Roberts & Cushman, LLP, Conlin, David G.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 2266
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6117645 20000912 <--
AB Disclosed are CHO cells which are capable of continued production of human LH-RH receptor **proteins**, or cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or partial peptides thereof; methods for screening compounds which have affinity for an LH-RH receptor by contacting the compound with the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the partial peptides thereof; kits for screening them; the compounds which have affinity for the LH-RH receptor obtained by. . .
SUMM The present invention relates to Chinese Hamster Ovary (CHO) cells having ability to continue producing human LH-RH (luteinizing hormone-releasing) receptor **proteins**, or cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or peptide fragments thereof; methods for screening a compound or a salt thereof which has affinity for an LH-RH receptor by using the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the peptide fragments thereof; kits for screening a compound or a salt thereof which has affinity for an LH-RH. . .
SUMM . . . is therefore considered that COS7 cells are unsuitable for screening use. Use of human pituitary fractions as human LH-RH receptor **protein** have been considered. However, human-derived tissues are very difficult to be obtained, resulting-in unsuitableness for screening use.
SUMM . . . is to provide CHO cells having ability to express human LH-RH receptor, cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or peptide fragments thereof; methods for screening a compound or a salt thereof which has affinity for an LH-RH receptor by using the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the peptide fragments thereof; kits for screening compounds or a salt thereof which has affinity for an LH-RH receptor.. . .
SUMM (1) A CHO cell containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA, and wherein said cell is capable of continued production of a recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**;
SUMM (2) A CHO cell containing a recombinant human LH-RH receptor **protein**, which is produced by cultivating the CHO cell described in (1) under conditions such that the recombinant human LH-RH receptor **protein** is continuously expressed from a DNA coding for a human LH-RH receptor **protein**, or a cell membrane fraction thereof;

SUMM (4) A recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which is isolated from the CHO cell described in (2);

SUMM (5) A method for producing a recombinant human LH-RH receptor **protein**, which comprises cultivating the CHO cell described in (1) under conditions suitable for expression of the recombinant human LH-RH receptor, continuously expressing a-recombinant human LH-RH receptor **protein** from said DNA and wherein said cell is capable of continued production of a recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**;

SUMM . . . salt thereof which has affinity for an LH-RH receptor, which comprises contacting the compound with the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof described in (4) and measuring the affinity of said compound for the. . .

SUMM . . . which contains the CHO cell or the cell membrane fraction thereof described in (.2), or the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof described in (4);

SUMM (22) The CHO cell described in (1) or (2), in which the DNA coding for the human LH-RH receptor **protein** is a DNA containing a DNA fragment having a nucleotide sequence represented by SEQ ID NO: 1;

SUMM (23) The recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof described in (4), in which said recombinant human LH-RH receptor **protein** is a **protein** having an amino acid sequence represented by SEQ ID NO: 2, an amino acid sequence lacking one amino acid or. . . amino acid sequence represented by SEQ ID NO: 2 are substituted by another amino acid or other amino acids, a **protein** in which an N-terminal signal peptide of said **protein** is removed, a **protein** in which a side chain of an amino acid in a molecule of said **protein** is protected with an appropriate protective group (for example, a C.sub.1-6 acyl group such as formyl or acetyl), or a **protein** in which a sugar chain is bound to said **protein** ;

SUMM . . . generation of intracellular CAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of guanine nucleotide refulatory **proteins** (G **proteins**) and cell growth);

SUMM . . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of guanine nucleotide refulatory **proteins** (G **proteins**) and cell growth);

SUMM (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a ligand to an LH-RH receptor, and

SUMM (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a ligand to an LH-RH receptor and a. . .

SUMM (i) contacting the recombinant human LH-RH receptor **protein**, a

peptide fragment thereof or a salt thereof described in (4) with a labeled ligand to an LH-RH receptor, and

SUMM (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a labeled ligand to an LH-RH receptor and. . .

SUMM (30) A vector containing a DNA coding for a human LH-RH receptor **protein** which expresses a human LH-RH receptor **protein** , which is designated pA1-11/hLH-RHR contained in Escherichia coli MV1184//pA1-11/hLH-RHR(FERM BP-4645, IFO 15812);

SUMM . . . CHO cell described in any one of (1)-(3) and (20)-(22), in which the DNA coding for the human LH-RH receptor **protein** is the expression vector described in (30);

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which comprises adaptating the CHO cell described in (1) to an agitation culture in suspension by use of a serum-containing. . .

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which comprises adaptating the CHO cell described in (1) to an agitation culture in suspension by use of a serum-containing. . .

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which comprises adaptating the CHO cell described in (1) to a serum-free medium with a gradual decrease in serum concentration. . .

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which comprises adaptating the CHO cell described in (1) to a serum-free culture in static culture (for example, plate culture),. . .

SUMM (36) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and wherein said cell is capable of continued production of the recombinant receptor **protein**

having activities substantially equivalent to those of the natural receptor **protein** to an agitation culture in suspension by use of a serum-containing medium;

SUMM (37) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, followed by adaptating to a serum-free medium, or

SUMM (38) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises adaptating a cell containing the DNA coding for the human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant human-derived receptor **protein** having activities substantially equivalent to those of the natural human-derived receptor **protein** to a serum-free medium with a gradual decrease in serum concentration under agitation culture in suspension;

SUMM (39) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to a serum-free culture in static culture (for example, plate culture), followed by adaptating to an agitation culture in suspension;. . .

SUMM . . . capable of proliferation in suspension produced by the method described in (36)-(39), which contains a DNA coding for a receptor **protein**, constitutively expresses a recombinant receptor **protein** from said DNA and has ability to keep producing a recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**.

SUMM As used herein, the "recombinant human LH-RH receptor **protein**" is a **protein**, mutein or peptide fragment having biological activities substantially equivalent to those of the natural human LH-RH receptor **protein**. Substantially equivalence will depend on the particular activity one is looking at. Biological activities include, for example, ligand binding and. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell

growth.

DRWD FIG. 1 shows a nucleotide sequence of cDNA coding for a human LH-RH receptor **protein** prepared in Reference Example 2;

DRWD FIG. 3 is a schematic representation showing the construction of a human LH-RH receptor **protein** expression vector designated pA1-11/hLH-RHR, wherein Amp.sup.r represents an ampicillin resistant gene, DHFR represents a dihydrofolate reductase gene, and SV40ori represents. . .

DETD The CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA, wherein said cell is capable of continued production of the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** is the CHO cell into which the expression vector containing the DNA coding for the human LH-RH receptor **protein** is introduced.

DETD As the DNA coding for the human LH-RH receptor **protein**, for example, cDNA or genomic DNA coding for the human LH-RH receptor **protein** is used. However, it is not necessarily limited thereto as long as it has a nucleotide sequence coding for the human LH-RH receptor **protein** or a peptide fragment thereof having ligand binding activities substantially equivalent to those of the human LH-RH receptor **protein**. For example, although known cDNA or genomic DNA coding for the human LH-RH receptor **protein** can be used, synthetic DNA may also be used. Examples thereof include DNA having the nucleotide sequence represented by SEQ. . . of the 54th to 1037th nucleotides of the nucleotide sequence shown in FIG. 1) coding for a human LH-RH receptor **protein** having the amino acid sequence represented by SEQ ID NO: 2 (FIG. 2). Specifically, CDNA having the nucleotide sequence of. . .

DETD In order to introduce the DNA fragment coding for the human LH-RH receptor **protein** into the CHO cell to express the recombinant human LH-RH receptor **protein**, it is necessary to construct the expression vector.

DETD . . . which the above-mentioned promoter (particularly, the SR.alpha. promoter) is inserted upstream from the DNA coding for the human LH-RH receptor **protein**, the polyadenylation signal is inserted downstream from the DNA coding for the human LH-RH receptor **protein**, further, the above-mentioned promoter (particularly, the SV40 promoter), the DHFR gene and/or the polyadenylation signal is inserted downstream therefrom, and. . .

DETD . . . preferred in which the SV40ori and SR.alpha. promoters are inserted upstream from the DNA coding for the human LH-RH receptor **protein** in vector pA1-11, the polyadenylation signal is inserted downstream from the DNA coding for the human LH-RH receptor **protein**, further, the SV40 promoter, the DHFR gene and the polyadenylation signal in this order are inserted downstream therefrom, and the. . .

DETD . . . can also be used. The animal cells may be any as long as they can express the human LH-RH receptor **proteins**. Examples thereof include 293 cells, Vero cells, L cells, myeloma cells, C127 cells, BALB3T3 cells and Sp-2/O cells. Of these,. . .

DETD The CHO cell containing the recombinant human LH-RH receptor **protein** of the present invention can be produced by cultivating the CHO cell containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA, wherein said cell is capable of continued production of the recombinant human LH-RH receptor

protein having activities substantially equivalent to those of the natural human LH-RH receptor **protein,** under such conditions that the DNA coding for the human LH-RH receptor **protein** can be constitutively expressed.

DETD Methods for stably expressing the human LH-RH receptor **proteins** using the CHO cells described above include methods of selecting the CHO cells by clone selection in which the above-mentioned. . . .

DETD . . . using the selection markers make it possible to obtain stable cell lines having high expression of the human LH-RH receptor **proteins**. Furthermore, when the DHFR genes are used as the selection marker, cultivation can also be performed with a gradual increase. . .

DETD . . . method for producing a CHO cell capable of proliferation in suspension containing a DNA coding for a human LH-RH receptor **protein,** constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA, wherein said cell is capable of continued production of the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein,** which comprises

DETD (1) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein,** constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, or

DETD (2) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein,** constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, followed by adaptating to a serum-free medium, or

DETD (3) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein,** constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to a serum-free medium with a gradual decrease in serum concentration under an agitation culture in suspension, or

DETD (4) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein,** constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to a serum-free culture in static culture (for example, plate culture), followed by adaptating to an agitation culture in suspension.

DETD . . . selection drugs such as MTX to make them selection drug-resistant, thereby amplifying the structural genes of the human LH-RH receptor **proteins**, or to improve productivity at the line level by combining them.

DETD Using the thus-obtained highly-productive CHO cell lines for the human LH-RH receptor **proteins**, large-scale cultivation is conducted to produce the target human LH-RH receptor **proteins** in large amounts. Culture apparatuses used in this case include known agitation

culture tanks equipped with elements necessary for cultivation. . . means as so desired [Shin Seikagaku Jikken Koza (Course of Biochemical Experiments, New Series), 1, edited by Nippon Seikagaku Kai, **Proteins** VI, Synthesis and Expression, pages 282 and 286, Tokyo Kagaku Dojin (1992); Shin Seikagaku Jikken Koza (Course of Biochemical Experiments,. . .

DETD The cell containing the recombinant human LH-RH receptor **protein** can be produced from the cell containing the expression vector bearing the DNA coding for the human LH-RH receptor **protein** in the manner as described above.

DETD Examples of the cells which can highly express the DNAs coding for the human LH-RH receptor **proteins** in the present invention include the CHO(dhfr.sup.-) cell containing the expression vector designated pA1-11/hLH-RHR which is obtained in Example 1. . . . CHO/L39-7 is preferred. Further, examples of the cells which can highly express the DNAs coding for the human LH-RH receptor **proteins** and can be suspension cultivated include CHO(dhfr.sup.+) cells designated CHO/LS and CHO/LH-8. Of these, the CHO(dhfr.sup.+) cell designated CHO/LH-8 is.

. .

DETD . . . CHO(dhfr.sup.-) cells have receptor activities (for example, ligand binding activity) about 10 times higher than the recombinant human LH-RH receptor **protein**-containing COS-7 cells. Expression of receptor in COS-7 cells is transient but expression of receptor in CHO cells is continuous. Accordingly,. . .

DETD . . . for producing the CHO cells capable of proliferation in suspension can be applied not only to the human LH-RH receptor **proteins**, but also to all receptor **proteins**, and can be applied not only to the CHO cells, but also to all cells.

DETD (1) a method for producing a cell capable of proliferation in suspension containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing a recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises

DETD (i) adaptating a cell containing the DNA coding for the human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant human-derived receptor **protein** having activities substantially equivalent to those of the natural human-derived receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, or

DETD (ii) adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant human-derived receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, followed by adaptating to a serum-free medium, or

DETD (iii) adaptating a cell containing the DNA coding for the human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to a serum-free medium with a gradual decrease in serum concentration under an agitation culture in suspension, or

DETD (iv) adaptating a cell containing the DNA coding for the receptor

protein, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to a serum-free culture in static culture (for example, plate culture), followed by adaptating to an agitation culture in suspension;. . .

DETD . . . capable of proliferation in suspension produced by the method described in (1), which contains a DNA coding for a receptor **protein**, constitutively expresses a recombinant receptor **protein** from said DNA and has ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**.

DETD The receptor **proteins** are not limited to the human LH-RH receptor **proteins**. They may be either known or novel receptor **proteins**. Examples thereof include endothelin receptor **proteins**, TRH receptor **proteins**, PACAP receptor **proteins**, histamine receptor **proteins**, somatostatin receptor **proteins**, CRF receptor **proteins**, neurotensin receptor **proteins**, IL-8 receptor **proteins**, galanin receptor **proteins**, GHRH receptor **proteins**, prostaglandin E.sub.2 receptor **proteins**, prostaglandin I.sub.2 receptor **proteins**, bradykinin receptor **proteins**, CNP receptor **proteins**, CC chemokine receptor **proteins**, angiotensin receptor **proteins**, bombesin receptor **proteins**, kanabinoid receptor **proteins**, cholecystokinin recetpor **proteins**, glutamine receptor **proteins**, serotonin receptor **proteins**, melatonin receptor **proteins**, neuropeptide Y receptor **proteins**, opioid receptor **proteins**, purine receptor **proteins**, vasopressin receptor **proteins**, oxytocin receptor **proteins**, VIP (Vasoactive intestinal and related peptide) receptor **proteins**, dopamine receptor **proteins**, motilin receptor **proteins**, amylin receptor **proteins**, bradykinin receptor **proteins**, CGRP (calcitonin gene related peptide) receptor **proteins**, leukotriene receptor **proteins**, pancreastatin receptor **proteins**, thromboxane receptor **proteins**, adenosine receptor **proteins**, adrenalin receptor **proteins**, GRO.alpha. receptor **proteins**, GRO.beta. receptor **proteins**, GRO.gamma. receptor **proteins**, NAP-2 receptor **proteins**, ENA-78 receptor **proteins**, PF-4 receptor **proteins**, IP10 receptor **proteins**, GCP-2 receptor **proteins**, MCP-1 receptor **proteins**, HC14 receptor **proteins**, MCP-3 receptor **proteins**, I-309 receptor **proteins**, MIP1.alpha. receptor **proteins**, MIP-1.beta. receptor **proteins**, RANTES receptor **proteins**, enterogastrine receptor **proteins**, pancreatic polypeptide receptor **proteins** and adrenomedulin receptor **proteins**.

DETD The DNAs coding for these receptor **proteins** can be cloned by methods well known in the art or methods based thereon. Expression vectors containing the DNAs can. . . methods based thereon, specifically according to methods for constructing expression vectors containing the DNAs expressing the above-mentioned human LH-RH receptor **proteins**.

DETD . . . the CHO cells. Any cells may be used as long as they can express the DNAs coding for the receptor **proteins** (preferably, human-derived receptor **proteins**) and can produce the recombinant receptor **proteins** having activities substantially equivalent to those of the natural receptor **proteins**. For

example, they include Escherichia, Bacillus, yeast, insects and animal cells.

DETD . . . proliferation in suspension, methods for cultivating the cells capable of proliferation in suspension and methods for isolating the recombinant receptor **proteins** produced, methods similar to those used for the above-mentioned human LH-RH receptor **proteins** can be employed.

DETD The cell membrane fraction of the cell (for example, the CHO cell) containing the recombinant human LH-RH receptor **protein** of the present invention means a fraction rich in the cell membrane content which is obtained by methods well known in the art after disruption of the cell containing the recombinant human LH-RH receptor **protein** of the present invention. Methods for disrupting the cell include crushing of the cell with a homogenizer and disruption with. . . as a membrane fraction. The membrane fraction contains a large amount of membrane components such as the human LH-RH receptor **protein,** cellderived phospholipids and membrane **proteins.**

DETD The amount of the human LH-RH receptor **proteins** in the cells containing the recombinant human LH-RH receptor **proteins** of the present invention or the cell membrane fractions thereof is preferably about 0.01 to about 100 pmol per 1 mg of the membrane **protein,** or preferably 10.sup.3 to 10.sup.8 molecules per cell, and more preferably 10.sup.4 to 10.sup.6 molecules per cell. The larger expression. . .

DETD Examples of the recombinant human LH-RH receptor **proteins** of the present invention include the recombinant human LH-RH receptor **protein** having the amino acid sequence represented by SEQ ID NO: 2 which is produced by expressing the DNA having the nucleotide sequence represented by SEQ ID NO: 1. They further include the **protein** having the amino acid sequence lacking one amino acid or two or more amino acids in the amino acid sequence represented by SEQ ID NO: 2, the **protein** having the amino acid sequence in which one amino acid or two or more amino acids are added to the. . . ID NO: 2 are substituted by another amino acid or other amino acids. Further, in these recombinant human LH-RH receptor **proteins,** N-terminal signal peptides may be cleaved, side chains of amino acids in molecules may be protected with appropriate protective groups (for example, C.sub.1-6 acyl groups such as formyl and acetyl), or sugar chains may be bound to the **proteins.**

DETD Recombinant human LH-RH receptor **proteins** of the present invention may be different from the known human LH-RH receptor **proteins** such as natural human LH-RH receptor **protein,** a recombinant human LH-RH receptor **protein** produced by cultivating COS-7 cells containg a DNA coding for human LH-RH receptor **protein,** in the kind, size and/or numbers of the glycosyl chains. Thus, the molecular weight of the recombinant human LH-RH receptor **protein** may be different from the molecular weight of the known human LH-RH receptor **proteins.**

DETD As the salts of the recombinant human LH-RH receptor **proteins** of the present invention, pharmaceutically acceptable acid addition salts are preferred among others. Examples of such salts include salts with. . .

DETD The recombinant human LH-RH receptor **protein** of the present invention can be produced, for example, by cultivating the CHO cell of the present invention containing the vector bearing the DNA coding for the human LH-RH receptor **protein,** under such conditions that the DNA coding for the human LH-RH receptor **protein** can be

expressed. The recombinant human LH-RH receptor **protein** can be isolated from the resulting cell containing the recombinant human LH-RH receptor **protein**, for example, according to the following methods.

DETD When the recombinant human LH-RH receptor **protein** is extracted from the cells, the cells are collected by known methods after cultivation, and suspended in an appropriate buffer. . . a homogenizer or freeze-thawing, followed by centrifugation or filtration to obtain a crude extract of the recombinant human LH-RH receptor **protein**.

DETD . . . such as CHAPS, digitonin or Triton X-100 (registered trade mark, hereinafter occasionally abbreviated as "TM"). The recombinant human LH-RH receptor **protein** contained in the resulting extract can be purified by suitable combinations of the separating-purifying methods well known in the art.. . .

DETD When the recombinant human LH-RH receptor **proteins** thus obtained are free forms, they can be converted to appropriate salts by known methods or methods based thereon. Conversely, when the **proteins** are obtained in the salt state, they can be converted to the free forms or other salts by known methods. . .

DETD Before or after purification, the recombinant human LH-RH receptor **protein** can be modified with an appropriate **protein** modifying enzyme to arbitrarily modify the **protein** or to partially eliminate a polypeptide therefrom. The **protein** modifying enzymes used include trypsin, chymotrypsin, arginyl endopeptidase, **protein** kinase and glucosidase.

DETD The recombinant human LH-RH receptor **protein** produced by cultivating the CHO cell containing the vector bearing the DNA coding for the human LH-RH receptor **protein**, under the conditions that the DNA coding for the human LH-RH receptor **protein** can be expressed, as described above, has activities substantially equivalent to those of the natural human LH-RH receptor **protein**. The substantially equivalent activities include, for example, ligand binding activity and signal information transmission. The ligand binding activity includes binding. . . LH-RH), LH-RH receptor superagonist (e.g. leuprorelin, leuprorelin acetate) or LH-RH receptor antagonist. As used herein, the "recombinant human LH-RH receptor **protein**" is a **protein**, mutein or peptide fragment having biological activities substantially equivalent to those of the natural human LH-RH receptor **protein**. Substantially equivalence will depend on the particular activity one is looking at. Biological activities include, for example, ligand binding and. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth. The ligand binding activity includes binding with, for example, LH-RH receptor agonist (e.g. LH-RH), LH-RH receptors superagonist (e.g. leuprorelin, leuprorelin acetate) or LH-RH receptor antagonist. Preferably, the recombinant human LH-RH receptor **protein** will have at least two of these activities, most preferably at least three. In addition, the recombinant receptor **protein** will have at least 50% of the activity of the natural human LH-RH receptor **protein**, preferably at least 70%, most preferably at least 90%. Accordingly, quantitative factors such as the molecular weight of the receptor **protein** may be different.

DETD As the peptide fragments of the recombinant human LH-RH receptor **proteins** of the present invention, for example, a site exposed

outside the cell membranes is used. Specifically, the peptide fragment is. . .

DETD As the salts of the peptide fragments of the recombinant human LH-RH receptor **proteins** of the present invention, pharmaceutically acceptable acid addition salts are preferred among others. Examples of such salts include salts with. . .

DETD The peptide fragments of the recombinant human LH-RH receptor **proteins** of the present invention or a salt thereof can be produced by peptide synthesis well known in the art or by cleaving the recombinant human LH-RH receptor **proteins** of the present invention with appropriate peptidases. For example, either solid phase synthesis methods or liquid phase synthesis methods may. . . the peptides. Namely, the target peptides can be produced by condensing peptide fragment(s) or amino acid(s) which can constitute the **proteins** of the present invention with residual moieties, and eliminating protective groups when the products have the protective groups. Known condensing. . .

DETD (4) H. Yazima, S. Sakakibara et al., Seikagaku Jikken Koza (Course of Biochemical Experiments), 1, Chemistry of **Proteins** IV, 205 (1977); and

DETD . . . by the above-mentioned methods are free forms, they can be converted to appropriate salts by known methods. Conversely, when the **proteins** are obtained in the salt state, they can be converted to the free forms by known methods.

DETD The CHO cells containing the recombinant human LH-RH receptor **proteins** or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins**, the peptide fragments thereof or a salt thereof according to the present invention is useful for screening a compound or. . .

DETD (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a ligand to an LH-RH receptor, and

DETD (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a ligand to an LH-RH receptor and. . .

DETD . . . to an LH-RH receptor with the CHO cell or the cell membrane fraction thereof containing the recombinant human LH-RH receptor **protein**, and

DETD . . . and a test compound with the CHO cell or the cell membrane fraction thereof containing the recombinant human LH-RH receptor **protein**.

DETD . . . the present invention comprises measuring the binding of a ligand to an LH-RH receptor to the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof, or the CHO cell containing the recombinant human LH-RH receptor **protein** or the cell membrane fraction thereof, for both the cases of (i) and (ii), or measuring cell stimulation activities, followed. . .

DETD (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a labeled ligand to an LH-RH receptor,. . .

DETD (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a labeled ligand to an LH-RH receptor. . .

DETD (i) contacting the CHO cells or membrane fractions thereof containing a recombinant human LH-RH receptor **protein** of the present invention with a labeled ligand to an LH-RH receptor, and

DETD (ii) contacting the CHO cells or membrane fractions thereof containing a

recombinant human LH-RH receptor **protein** of the present invention with a labeled ligand to an LH-RH receptor and a test compound;

DETD . . . salt thereof, which comprises contacting the CHO cell or the cell membrane fraction thereof containing a recombinant human LH-RH receptor **protein** of the present invention with a test compound;

DETD . . . generation of intracellular CAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth);

DETD (i) contacting the CHO cell or the cell membrane fraction thereof containing a recombinant human LH-RH receptor **protein** of the present invention with an LH-RH receptor agonist, and

DETD (ii) contacting the CHO cell or the cell membrane fraction thereof containing a recombinant human LH-RH receptor **protein** of the present invention with an LH-RH receptor agonist and a test compound,

DETD . . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth).

DETD In the above-mentioned screening method (1a) or (2a), a compound which binds to the recombinant human LH-RH receptor **protein** or a peptide fragment thereof or the CHO cell or a membrane fraction thereof of the present invention inhibits the binding of a ligant to an LH-RH receptor with the recombinant human LH-RH receptor **protein** can be selected as the compound or a salt thereof which has affinity for an LH-RH receptor.

DETD Further, in the above-mentioned screening method (2b), a compound which binds to the human LH-RH receptor **protein** to exhibit cell stimulation activities through the human LH-RH receptor (for example, activities enhancing or inhibiting arachidonic acid release, acetylcholine. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growht) can be selected as a human LH-RH receptor agonistic compound. Of the LH-RH receptor agonistic compounds, compounds. . .

DETD . . . binding of an LH-RH receptor agonist to CHO cell or a membrane fraction thereof containing the recombinant human LH-RH receptor **protein** but does not have the cell stimulation activities can be selected as the human LH-RH receptor antagonistic compound.

DETD Prior to the acquisition of the CHO cells containing the recombinant human LH-RH receptor **proteins** of the present invention, there were no animal cells capable of highly expressing the recombinant human LH-RH receptor **proteins**. It was therefore impossible to efficiently screen compounds or a salt thereof which have affinity for the LH-RH receptor, especially. . . the CHO cells introduced by the human LH-RH receptor CDNA of the present invention can express the human LH-RH receptor **proteins** in large amounts, so that they are useful for the screening of the compounds which have affinity for the LH-RH. . . capable of proliferation in suspension of the present invention are suitable for large-scale cultivation of the recombinant human LH-RH receptor **proteins**.

DETD When the CHO cells expressing the human LH-RH receptor **proteins** are used in the screening methods of the present invention, the CHO cells can be fixed with glutaraldehyde, formalin, etc.. . .

DETD Examples of the test compounds include peptides, **proteins**, non-peptide compounds, synthetic compounds, fermented products, cell extracts, plant extracts and animal tissue extracts, which may be either novel compounds. . .

DETD Specifically, when the above-mentioned screening method (1a) or (2a) is conducted, the CHO cell containing the recombinant human LH-RH receptor **protein** or the cell membrane fraction thereof, or the recombinant human LH-RH receptor **protein** or the peptide fragment thereof according to the present invention is first suspended in a buffer solution suitable for screening. . .

DETD . . . when the above-mentioned screening method (2b) and (2c) are conducted, the cell stimulation activities through the recombinant human LH-RH receptor **protein** (for example, activities enhancing or inhibiting arachidonic acid release, acetylcholine release, fluctuation in intracellular Ca.sup.2+ concentration, generation of intracellular cAMP, generation of intracellular cGXP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and secretion of hormones) and cell growth can be assayed by known methods or by use of commercial measuring kits. Specifically, the CHO cell containing the recombinant human LH-RH receptor **protein** is first cultivated on a multiwell plate. In conducting the screening, the medium is preliminarily replaced by a fresh medium. . . a substance (for example, arachidonic acid) used as an indicator for the cell stimulation activities is difficult because of a **catabolic** enzyme contained in the cell, an inhibitor to the **catabolic** enzyme may be added to conduct the assay. Further, activity such as inhibition of cAMP production can be detected as. . .

DETD The kit for screening of the present invention contains the CHO cell containing the recombinant human LH-RH receptor **protein** or the cell membrane fraction thereof, or the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof according to the present invention.

DETD (I) Kits for Screening Containing the CHO Cell Containing the Recombinant Human LH-RH Receptor **Protein**

DETD (2) Recombinant Human LH-RH Receptor **Protein** Sample

DETD A sample obtained by cultivation of CHO cells (5.times.10.sup.4 cells/well) expressing a recombinant human LH-RH receptor **protein** in a 24-well plate at 37.degree. C. at 5% CO.sub.2 and 95% air for 2 days.

DETD (1) The CHO cells expressing the recombinant human LH-RH receptor **proteins** cultivated on the 24-well plate are washed once with 300 .mu.l of the assay buffer A, followed by addition of. . .

DETD (2) Recombinant Human LH-RH Receptor **Protein** Sample

DETD A sample is membrane fraction of CHO cells expressing human LH-RH receptor **protein**. Samples can be prepared from the CHO cells described above and stored at -80.degree. C. prior to use.

DETD (1) The membrane fraction of CHO cells expressing human LH-RH receptor **protein** is diluted to an appropriate concentration (about 0.1 to 5000 .mu.g/ml, preferably about 1 to 500 .mu.g/ml), and dispensed each. . .

DETD . . . kits for screening of the present invention are compounds inhibiting the binding of LH-RH to the recombinant human LH-RH receptor **proteins** of the present invention. The compounds are selected

from test compounds such as peptides, **proteins**, non-peptide compounds, synthetic compounds, cell extracts, plant extracts and animal tissue extracts which may be novel or known. The compounds. . .

DETD . . . receptor and therefore they are also useful as a recomposition to detect or assay a receptor expression cell, LH-RH receptor **protein** in a body.

DETD The CHO cells of the present invention wherein said cell is capable of continued production of the human LH-RH receptor **proteins** are cells capable of highly expressing the human LH-RH receptor **proteins**. In particular, the cells adapted to the CHO cells capable of proliferation in suspension are suitable for large-scale cultivation of the human LH-RH receptor **proteins**.

DETD . . . which have affinity for an LH-RH receptor using the CHO cells having ability to continue producing the human LH-RH receptor **proteins** or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the peptide fragments thereof according to the present invention, the LH-RH receptor agonistic, superagonistic or antagonistic compounds can be. . .

DETD [SEQ NO:1] Shows a nucleotide sequence of a cDNA coding for human LH-RH receptor **protein**.

DETD [SEQ NO:2] Shows an amino acid sequence of human LH-RH receptor **protein**.

DETD . . . of a DNA oligomer for PCR which is used for a cloning of a cDNA coding for rat LH-RH receptor **protein**. This sequence is a partial nucleotide sequence of a cDNA coding for murine LH-RH receptor **protein**.

DETD . . . of a DNA oligomer for PCR which is used for a cloning of a cDNA coding for rat LH-RH receptor **protein**. This sequence is a partial nucleotide sequence of a cDNA coding for murine LH-RH receptor **protein**.

DETD . . . LH-RH receptor cDNA expression vector. This sequence contains a partial nucleotide sequence of a cDNA coding for human LH-RH receptor **protein**.

DETD . . . LH-RH receptor cDNA expression vector. This sequence contains a partial nucleotide sequence of a cDNA coding for human LH-RH receptor **protein**.

DETD . . . 2 hours, and then, concentrated to obtain a residue, which was distributed between ethyl acetate and an aqueous solution of **ammonium chloride**. The aqueous layer was extracted with ethyl acetate. The extracts were collected and washed with saline. After drying on MgSO.sub.4,. . .

DETD . . . the single cell by the limiting dilution method to obtain cell line CHO/L39 which stably expresses the human LH-RH receptor **protein**. This cell line was repeatedly cloned to obtain cell line CHO/L39-7 which expresses the receptor in higher amount. In this. . . makes it possible to obtain a cell line in which an introduced gene is amplified and which expresses the desired **protein** with higher amount.

DETD The LH-RH receptor **protein** activity of the CHO cells or the cell membrane fraction thereof was assayed by the following method:

DETD Cell line CHO/L39-7 (2.times.10.sup.7 cells) highly expressing the human LH-RH receptor **protein** obtained in Example 2 were cultivated in 100 ml of TE medium [a 1:1 (v/v) mixed medium of Daigo T. . .

DETD . . . SEQUENCE CHARACTERISTICS:
(A) LENGTH: 328 amino - #acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- - (ii) MOLECULE TYPE: **protein**
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
- - Met Ala Asn Ser Ala Ser Pro Glu Gln Asn. . .

CLM What is claimed is:
1. A method for screening a compound or a salt thereof which has affinity for an LH-RH receptor **protein** comprising the amino acid sequence represented by SEQ ID NO:2, which comprises contacting the compound with the CHO cell line. . . cell line CHO/LH-8 or the cell membrane fraction thereof, and measuring the afinity of said compound for the LH-RH receptor **protein**.

2. A kit for screening a compound or a salt thereof which has affinity for an LH-RH receptor **protein**, comprising the amino acid sequence represented by SEQ ID NO: 2, which is expressed in the CHO cell line CHO/L39-7,. . .

L15 ANSWER 10 OF 109 USPATFULL

AB The present invention relates to a method for producing plants with improved agronomic and nutritional traits. Such traits include enhanced nitrogen assimilatory and utilization capacities, faster and more vigorous growth, greater vegetative and reproductive yields, and enriched or altered nitrogen content in vegetative and reproductive parts. More particularly, the invention relates to the engineering of plants modified to have altered expression of key enzymes in the nitrogen assimilation and utilization pathways. In one embodiment of the present invention, the desired altered expression is accomplished by engineering the plant for ectopic overexpression of one of more the native or modified nitrogen assimilatory enzymes. The invention also has a number of other embodiments, all of which are disclosed herein.

AN 2000:110050 USPATFULL
TI Transgenic plants that exhibit enhanced nitrogen assimilation
IN Coruzzi, Gloria M., New York, NY, United States
Brears, Timothy, Durham, NC, United States
PA New York University, New York, NY, United States (U.S. corporation)
PI US 6107547 20000822 <--
AI US 1997-987237 19971209 (8)
RLI Continuation of Ser. No. US 1994-319176, filed on 6 Oct 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-132334, filed on 6 Oct 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: McElwain, Elizabeth F.
LREP Pennie & Edmonds LLP
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 2487
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6107547 20000822 <--
SUMM . . . asparaginase (ANS; E.C. 3.5.1.1) to produce aspartate and ammonia which then could be utilized in synthesis of amino acids and **proteins** (See FIG. 1).

SUMM . . . Biol. 20:207-218 (transgenic tobacco plants overexpressing soybean GS in tobacco plants). One study has reported observing increases in total soluble **protein** content in transgenic tobacco plants overexpressing the alfalfa GS1 gene. However, since this same study also reported similar increases in total soluble **protein** content in transgenic tobacco plants expressing antisense RNA to the GS1 gene, the relationship between GS1 expression and the increase in soluble **protein** appears unclear (Temple et al., 1993, Mol. Gen. Genet. 236:315-325). One clearly established effect of GS overexpression in plants is. . .

SUMM . . . pattern or level of the nitrogen assimilation or utilization enzyme, altered expression pattern or level of the corresponding mRNA or **protein**, altered nitrogen assimilation or utilization capacities, increased growth rate, enhanced vegetative yield, or improved reproductive yields (e.g., more or larger seeds or fruits). The screening of the engineered plants may involve enzymatic assays and immunoassays to measure enzyme/**protein** levels; Northern analysis, RNase protection, primer extension, reverse transcriptase/PCR, etc. to measure mRNA levels; measuring the amino acid composition, free. . .

SUMM . . . operably linked with sequences encoding a pea glutamine synthetase (GS) gene or a pea asparagine synthetase (AS) gene. RNA and **protein** analyses showed that a majority of the engineered plants exhibited ectopic, overexpression of GS or AS. The GS or AS. . .

DRWD . . . and a sequence encoding a small subunit of a plant or E. coli NADH-GOGAT, containing the NADH-binding domain. The chimeric **protein** encodes a bispecific or bifunctional GOGAT enzyme which can utilize either Fd or NADH as the reductant.

DRWD FIG. 5. Analysis of GS **Protein** in Primary (T1) Transformants Containing GS Transgenes. Top panel: Western analysis of GS polypeptides in primary transformants. Lanes 1 and. . . are shown (as percentages relative to controls =(100%)) below the Western panel. Bottom panel: Coomassie staining of RUBISCO large subunit **protein** demonstrating approximately equal loading of samples. ctGS-chloroplastic GS2 (.about.45 kD); cyGS-cytosolic GS (.about.38 kD).

DRWD FIGS. 6A-C. Analysis of GS **Protein**, RNA and Holoenzyme from T2 Progeny Transgenic Plants Containing Pea GS Transgenes. Of the four T2 plants from each primary. . . Panel A (upper): Western analysis of GS polypeptides in transgenic plants. Panel A (lower): Coomassie staining of RUBISCO large subunit **protein** to show approximately equal loading of samples. Panel B (upper): Northern blots hybridized with the approximate cDNA probes for GS1. . .

DRWD FIG. 7A. Activity Gel Analysis of GS Holoenzymes. **Protein** extracts from pea chloroplast (PC), pea root (PR), tobacco chloroplast (TC) and tobacco roots (TR) demonstrating the migration of chloroplastic- and cytosolic-enriched GS **protein** samples relative to the migration of the holoenzymes of GS1 and GS3A overexpressing plants. Lane 1: pea chloroplast **protein** (PC) has GS holoenzyme B only; lane 2: pea root **protein** (PR) has GS holoenzyme C only; lane 3: tobacco chloroplast **protein** (TC) has GS holoenzyme B only; lane 4: tobacco root **protein** has GS holoenzyme C only. Lane 5: **protein** from plant Z17-7 (carrying

the 35S-GS3A construction) has GS holoenzymes A* and B; lane 5: **protein** from plant Z3-1 (carrying the 35S-GS1 construction) has GS holoenzymes B and C.

DRWD FIG. 7B. Western Analysis of GS **Proteins** Isolated from GS Holoenzymes A*, B, and C. Holoenzymes A* and C observed in transgenic tobacco overexpressing GS3A and GS1 were excised from non-denaturing gels, re-extracted in **protein** isolation buffer, and electrophoresed under denaturing conditions for Western analysis using GS antibodies. Lane 1: tobacco leaf **protein** as control; lane 2: GS holoenzyme A* from Z17-7; lane 3: isolated chloroplast GS2 (holoenzyme B) as control; lane 4:. . .

DRWD FIG. 8. Western and Northern Analysis of GS **Protein** and RNA in Transgenic Plants Selected for Growth Analysis Ectopically Expressing either Cytosolic GS1 or GS3A. Upper panel: Western blot for GS **proteins**. Lower panel: Northern blot for GS mRNA. Pl and Tl are pea and tobacco leaf controls. Lanes 1 and 2,. . .

DRWD FIGS. 11A and 11B. Linear relationship between GS activity and plant fresh weight or total leaf **protein**. T2 progenies of primary transformants which showed no segregation of the Kan.sup.R phenotype associated with the transgene were selected for. . . of total leaf

GS

as determined by the transferase assay (B. M. Shapiro, et al., Methods Enzymol. 17A:910 (1970)) and **protein**/gram fresh weight. Plants analyzed were: Control, SR1 untransformed tobacco; Z54-4 co-suppressed by GS2; Z17-7 overexpressing GS3A; Z3-1 overexpressing GS1. FIG. 11A; Plant fresh weight vs. GS activity. FIG. 11B; **protein**/gm fresh weight vs. GS activity.

DETD . . . present invention may involve engineering plants with ectopic overexpression of enzymes catalyzing the use of glutamine, glutamate

and

asparagine in **catabolic** reactions. In a preferred embodiment, a plant is engineered for the ectopic overexpression of asparaginase.

DETD . . . tissues and organs are desired, promoters such as those of the ribulose bisphosphate carboxylase (RUBISCO) genes or chlorophyll a/b binding **protein** (CAB) genes may be used; where expression in seed is desired, promoters such as those of the various seed storage **protein** genes may be used; where expression in nitrogen fixing nodules is desired, promoters such those of the legehemoglobin or nodulin. . .

DETD . . . heat shock genes, the defense responsive gene (e.g., phenylalanine ammonia lyase genes), wound induced genes (e.g., hydroxyproline rich cell wall **protein** genes), chemically-inducible genes (e.g., nitrate reductase genes, gluconase genes, chitinase genes, etc.), dark-inducible genes (e.g., asparagine synthetase gene (Coruzzi and. . .

DETD . . . 3) enzymatic assays for detecting enzyme or ribozyme activity, where such gene products are encoded by the gene construct; 4) **protein** gel electrophoresis, Western blot techniques, immunoprecipitation, or enzyme-linked immunoassays, where the gene construct products are **proteins**. Additional techniques, such as in situ hybridization, enzyme staining, and immunostaining, also may be used to detect the presence or. . .

DETD . . . for suppression of a target gene, transformed plants are examined for those expressing the target gene product (e.g., RNA or **protein**) at reduced levels in various tissues. The plants exhibiting the desired physiological changes, e.g., ectopic GS overexpression or GS suppression,. . .

DETD . . . herein means any one or any mix of the ammonium salts commonly used as plant nitrogen fertilizer, e.g., ammonium nitrate, **ammonium chloride**, ammonium sulfate, etc.

DETD . . . of the transformed plants may be for improved agronomic characteristics (e.g., faster growth, greater vegetative or reproductive yields, or improved **protein** contents, etc.), as compared to unengineered progenitor plants, when cultivated under nitrogen non-limiting growth conditions (i.e., cultivated using soils or. . .

DETD . . . plants engineered with the alterations in nitrogen assimilation or utilization processes may exhibit improved nitrogen contents, altered amino acid or **protein** compositions, vigorous growth characteristics, increased vegetative yields or better seed yields and qualities. Engineered plants and plant lines possessing such. . . amino acid content of the plant; 8) the free amino acid content of the fruit or seed; 9) the total **protein** content of the plant; and 10) the total **protein** content of the fruit or seed. The procedures and methods for examining these parameters are well known to those skilled. . .

DETD . . . than non-suppressed plants. (See Knight and Langston-Unkefer , Science 241:951-954). GS suppressed plants may also have altered amino acid or **protein** contents, making such plants useful in preparation of special dietary foods. Further, all the engineered plants disclosed herein may also. . .

DETD . . . herein show that constitutive overexpression of a heterologous GS subunit for cytosolic GS leads to increases in GS mRNA, GS **protein**, total GS activity, native GS holoenzyme, and, in one case, to the production of a novel GS holoenzyme. Transformed plants. . . significant growth advantage compared to wild type. They grow faster, attain a higher final fresh weight and have more soluble **proteins** than untransformed progenitor plants during the vegetative stage of their development. In some instances, however, overexpression of cytosolic GS and/or. . . GS gene (i.e., co-suppression). Such GS co-suppressed plants may show poorer growth characteristics, but may have altered amino acid and **protein** contents due to shunting of nitrogen into other nitrogen assimilation/metabolism pathways.

DETD 6.1.4. GS **Protein** and Enzyme Activity Analysis

DETD Soluble **proteins** were extracted from tobacco and pea leaf tissue as previously described (Tingey and Coruzzi, 1987, Plant Physiol. 84:366-373). **Proteins** were denatured and separated in 12% acrylamide by SDS-PAGE and electroblotted onto nitrocellulose. Western analysis was undertaken using the ProtoBlot. . .

DETD . . . primary transformants (FIG. 6, lanes 9-14). Western blot analysis of these plants confirmed the low abundance of the chloroplast GS2 **protein** (FIG. 6, panel A) and non-denaturing GS activity gel analyses confirmed the reduced abundance of the GS2 holoenzyme (FIG. 6,. . . of a pea GS2 transgene. In addition, the pea GS2 transgene was also silenced. Levels of cytosolic GS mRNA and **protein** were unaffected in these GS2 co-suppressed plants.

DETD . . . of Z17. Transformant Z17-12 is co-suppressed for GS enzyme activity (27% of wild-type) and both chloroplastic GS2 and cytosolic GS **proteins** are low (FIG. 5, lane 2) compared to wild-type tobacco (FIG. 5, lane TL). By contrast, transformant Z17-6 has elevated levels of total GS activity (127%) and increased levels of cytosolic GS

protein (FIG. 5, lane 1) compared to wild-type tobacco (FIG. 5, lane TL). Analysis of the T2 progeny of other independent transformants revealed additional transformants to be down-regulated for cytosolic GS **protein** (Z17-9B and Z17-10; FIG. 6, Panel A, lanes 6 and 7), while others had elevated levels of cytosolic GS (Z17-7. . . analysis (FIG. 6, panel A, lanes 3-5) and GS activity assays (Table 1). Non-denaturing GS activity gel analysis of soluble **proteins** from these Z17 transformants which overexpress cytosolic GS3A indicates the existence of a novel GS holoenzyme (band A*, FIG. 6,. . .

DETD . . . of these Z3 transformants are shown in FIG. 6. Both Z3-1 and Z3-2 show an increased abundance of cytosolic GS **protein** (FIG. 6, panel A, lanes 1 and 2) and this is reflected by the increased levels of GS mRNA (FIG.. . .

DETD . . . transgenic plants was repeated in non-denaturing activity gels including for comparison, lanes of pea root (PR) and tobacco root (TR) **protein** which are enriched for the cytosolic GS holoenzyme (band C) FIG. 7A, lanes 2 and 4), and extracts derived from. . . composition of the GS activity bands A*, B, and C, these bands were excised from preparative gels, and the extracted **proteins** were reloaded on a denaturing SDS gel followed by Western blot analysis for GS subunits (FIG. 7B). This analysis revealed. . . GS2 subunits. It is possible that GS activity band A* represents the association of transgenic GS3A subunits with a chaperonin-type **protein**, but attempts to dissociate such a complex with ATP were unsuccessful. Consequently, the nature of the novel GS holoenzyme remains. . .

DETD Plant growth analysis was undertaken on the T2 progeny plants analyzed for GS **protein** and RNA in FIG. 8. Individual T2 plants were grown in white sand and growth was assessed by fresh weight. . .

DETD 6.2.11. Correlation Between GS Activity and Final Fresh Weight and Total **Protein**

DETD . . . controls by 1.5-times and 2-times, respectively. For these same individual T2 plants, a linear relationship also exists between total leaf **protein** (.mu.g **protein**/gm fresh weight) and leaf GS activity. Plants expressing the highest levels of GS activity (284%) had 1.5-fold higher levels of soluble **protein**/gram fresh weight compared to controls (FIG. 11B). An unpaired T-test analysis of this data revealed that the GS overexpressing lines (Z3-1,Z17-7) had significantly greater GS activity, fresh weight, and leaf soluble **protein** with a p value of <0.0001, with the exception of fresh weight for Z17-7 whose p value was 0.0007. Similarly the line co-suppressed by GS2 (Z54-4) had significantly less GS activity, fresh weight, and leaf soluble **protein** than control SR1 with a p value of <0.0001. The GS activity profiles of the GS overexpressing T2 lines used. . .

DETD . . . homologs may be more complex than the overexpression of genes for which there are no homologs, such as viral coat **protein** and BT toxin genes (Powell-Abel et al., 1986, Science 232:738-743; Vaeck et al., 1987, Nature 328:33-37). This is due to. . . cytosolic GS which were successfully overexpressed (GS1 and GS3A), the overexpression resulted not only in over production of GS RNA, **protein** and enzyme, but also in a phenotype of improved nitrogen use efficiency.

DETD . . . of the pea gene for cytosolic GS1 in tobacco gives a clear phenotype of increased GS activity, increased cytosolic GS **protein**, and high levels of transgene mRNA. Furthermore, the GS1

protein assembles into a GS holoenzyme similar in size and charge to native pea cytosolic GS. In transgenic plants overexpressing cytosolic. . . of the overexpressed cytosolic subunits to be released from an assembling chaperonin. Indeed, the close association of GS with groEL-like **proteins** has previously been observed in pea (Tsuprun et al., 1992, Biochim. Biophys. Acta 1099:67-73). However, our attempts to dissociate the. . .

DETD . . . GS activity and an improvement in plant growth and nutritional characteristics. Temple et al. reported increases in GS mRNA and **protein**, but no corresponding increase in GS activity in the transgenic plants (Temple et al., ibid). Hemon et al. reported increased levels of GS mRNA in transgenic plants engineered with GS expression constructs, but found no corresponding increase in GS **protein** or enzyme activity (Hemon et al., ibid). In two other reports, overexpression of GS genes in transgenic plants did result. . .

DETD . . . unstable, the AS enzyme has never been purified to homogeneity and antibodies for plant AS were not available for AS **protein** analysis. In addition, in vitro assay detected no AS activity due to enzyme instability.

DETD . . . for nutrient availability and nitrogen is typically the most critical nutrient at this time due to the synthesis of new **proteins** in expanding and enlarging tissues. Nitrogen assimilated and accumulated at this time is subsequently recycled in the plant and deposited. . .

CLM What is claimed is:

. . . amino acid content in the whole plant, vii) greater free amino acid content in the fruit or seed, viii) greater **protein** content in seed or fruit, or ix) greater **protein** content in a vegetative tissue, than that of a progenitor plant which does not contain the gene construct, when the. . .

. . . amino acid content in the whole plant, vii) greater free amino acid content in the fruit or seed, viii) greater **protein** content in seed or fruit, or ix) greater **protein** content in a vegetative tissue, than a progenitor plant which does not contain the gene construct, when the transgenic plant. . .

L15 ANSWER 11 OF 109 USPATFULL

AB Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

AN 2000:98553 USPATFULL

TI Polypeptides altered to contain an epitope from the Fc region of an IgG molecule for increased half-life

IN Presta, Leonard G., San Francsico, CA, United States
Snedecor, Bradley R., Portola Valley, CA, United States

PA Genentech, Inc., S. San Francisco, CA, United States (U.S. corporation)

PI US 6096871 20000801 <--

AI US 1995-422093 19950414 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Reeves, Julie

LREP Hasak, Jan, Vance, Dolly A.Flehr Hohbach Test Albritton & Herbert LLP

CLMN Number of Claims: 14

ECL Exemplary Claim: 1

DRWN 4 Drawing Figure(s); 3 Drawing Page(s)

LN.CNT 3391
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6096871 20000801 <--
SUMM . . . (pFc') fragment of human IgG also produced by trypsin digestion
of the Fc fragment was rapidly eliminated, indicating that the **catabolic** site of IgG is located in the CH2 domain. Ellerson et al., J. Immunol., 116: 510 (1976); Yasmeen et al.,. . .
SUMM The **catabolic** rates of IgG variants that do not bind the high-affinity Fc receptor FcRI or C1q are indistinguishable from the rate of clearance of the parent wild-type antibody, indicating that the **catabolic** site is distinct from the sites involved in FcRI or C1q binding. Wawrzynczak et al., Molec. Immunol., 29: 221 (1992).. . .
SUMM Staphylococcal **protein** A-IgG complexes were found to clear more rapidly from the serum than uncomplexed IgG molecules. Dima et al.,
Eur. J.. . . on the pharmacokinetics of the Fc-hinge fragment. The authors showed that the site of the IgG1 molecule that controls the **catabolic** rate (the "**catabolic** site") is located at the CH2-CH3 domain interface and overlaps with the Staphylococcal **protein** A binding site. See also WO 93/22332 published Nov. 11, 1993. The concentration catabolism phenomenon is also studied in Zuckier. . .
SUMM WO 94/04689 discloses a **protein** with a cytotoxic domain, a ligand-binding domain and a peptide linking these two domains comprising
an IgG constant region domain having the property of increasing the half-life of the **protein** in mammalian serum.
SUMM A stereo drawing of a human Fc fragment and its complex with fragment B of **Protein** A from Staphylococcus aureus is provided by Deisenhofer, Biochemistry, 20: 2364 (1981).
DETD . . . as is well known to those skilled in the art of antibody technology. Examples of such polypeptides are peptides and **proteins**, whether from eukaryotic sources such as, e.g., yeast, avians, plants, insects, or mammals, or from bacterial sources such as, e.g.,. . .
DETD . . . hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as **Protein** C; atrial naturietic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or
tissue-type plasminogen activator (t-PA);. . . a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain;
relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial **protein**, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; **protein** A or D; rheumatoid factors; a neurotrophic factor such as brain-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3,. . . TGF-.beta.2, TGF-.beta.3, TGF-.beta.4, or TGF-.beta.5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding **proteins**; CD **proteins** such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic **protein** (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs),. . . IL-1 to IL-10; an anti-HER-2 antibody

without a native Fc region of an IgG; superoxide dismutase; T-cell receptors; surface membrane **proteins**; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport **proteins**; homing receptors; addressins; regulatory **proteins**; antibodies without a native Fc region of an IgG; and fragments of any of the above-listed polypeptides.

DETD Libraries are screened with probes designed to identify the gene of interest or the **protein** encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to. . .

DETD . . . In some preferred embodiments, the nucleic acid sequence includes the polypeptide of interest's signal sequence. Nucleic acid having all the **protein** coding sequence is obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for. . .

DETD . . . insertions include insertions to the internal portion of the polypeptide of interest, as well as N- or C-terminal fusions with **proteins** or peptides containing the desired epitope that will result, upon fusion, in an increased half-life.

DETD . . . bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for **protein** production, generally an expression vector of the type typically employed for transformation of an appropriate host.

DETD Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a **protein** necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode **proteins** that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or. . .

DETD . . . drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a **protein** conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et. . .

DETD . . . that encodes the polypeptide variant. Amplification is the process by which genes in greater demand for the production of a **protein** critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the. . .

DETD . . . cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the polypeptide variant, wild-type DHFR **protein**, and another selectable marker such as aminoglycoside 3-phosphotransferase (APH) can be selected by cell growth in medium containing a selection. . .

DETD . . . amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding **proteins** endogenous to the host, with examples of such hosts including E. coli W3110 strain 1A2, which has the complete genotype. . .

DETD . . . Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-**protein** duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound. . .

DETD . . . step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or

ultrafiltration; optionally, the **protein** may be concentrated
with a commercially available **protein** concentration filter,
followed by separating the polypeptide variant from other impurities by
one or more steps selected from immunoaffinity chromatography,. . .
chromatography on Blue-SEPHAROSE, CM Blue-BLUE-SEPHAROSG, MONO-Q,
MONO-S, lentil lectin-LENTIL LECTIN-SEPHAROSE, WGA-SEPHAROSE, CON
A-SEPHAROSE, ETHER, TOYOPEARL, BUTYL TOYOPEARL, PHENYL TOYOPEARL, or
protein A SEPHAROSE, SDS-PAGE chromatography, silica
chromatography, chromatofocusing, reverse phase HPLC (e.g., silica gel
with appended aliphatic groups), gel filtration using,. . .

DETD . . . another embodiment, supernatants from systems which secrete
recombinant polypeptide variant into culture medium are first
concentrated using a commercially available **protein**
concentration filter, for example, an Amicon or Millipore Pellicon
ultrafiltration unit. Following the concentration step, the concentrate
may be applied to a suitable purification matrix. For example, a
suitable affinity matrix may comprise a ligand for the **protein**
, a lectin or antibody molecule bound to a suitable support.
Alternatively, an anion-exchange resin may be employed, for example, a.
. . matrix or substrate having pendant DEAE groups. Suitable matrices
include acrylamide, agarose, dextran, cellulose, or other types
commonly
employed in **protein** purification. Alternatively, a
cation-exchange step may be employed. Suitable cation exchangers
include
various insoluble matrices comprising sulfopropyl or carboxymethyl
groups.. . .

DETD Mammalian polypeptide variant synthesized in recombinant culture is
characterized by the presence of non-human cell components, including
proteins, in amounts and of a character which depend on the
purification steps taken to recover the polypeptide variant from
culture.. . .

DETD . . . O-acetyl tyrosyl species and 3-nitro derivatives,
respectively.
Tyrosyl residues are iodinated using .sup.125 I or .sup.131 I to
prepare
labeled **proteins** for use in radioimmunoassay, the chloramine T
method described above being suitable.

DETD . . . of seryl or threonyl residues, methylation of the
.alpha.-amino
groups of lysine, arginine, and histidine side chains (T. E. Creighton,
Proteins: Structure and Molecular Properties, W. H. Freeman &
Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal
amine, and. . .

DETD . . . the compound tunicamycin as described by Duskin et al., J.
Biol. Chem., 257: 3105 (1982). Tunicamycin blocks the formation of
protein-N-glycoside linkages.

DETD . . . be fused to a second polypeptide and the antibody or fusion
thereof may be used to isolate and purify the **protein** to which
it binds from a source such as a CD11 or CD18 antigen. In another
embodiment, the invention provides. . .

DETD . . . as phosphate, citrate, and other organic acids; antioxidants
including ascorbic acid; low molecular weight (less than about 10
residues) polypeptides; **proteins**, such as serum albumin,
gelatin, or immunoglobulins; hydrophilic polymers such as
polyvinylpyrrolidone; amino acids such as glycine, glutamine,
asparagine, arginine. . .

DETD . . . (ip) injections of the relevant antigen and an adjuvant. It
may
be useful to conjugate the relevant antigen to a **protein** that

is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin. . .

DETD . . . the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different **protein** and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as **protein** fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

DETD . . . as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the **protein** used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable. . .

DETD . . . are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, **protein** A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DETD . . . E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin **protein**, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of. . .

DETD Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic **protein** chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a. . .

DETD . . . using leucine zippers. Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun **proteins** were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the. . .

DETD . . . tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or variant antibody, and **proteins** that bind to the analyte are denominated binding partners, whether they be antibodies, cell-surface receptors, or antigens.

DETD . . . flanking the F1 origin were removed from pB0475 and DNA coding for anti-CD18 Fab H52, version OZ (Eigenbrot et al., **Proteins**, 18: 49-62 [1994]) was substituted for DNA coding for human growth hormone using the EcoRV and SphI sites. Hence, pH52. . .

DETD . . . 0.3 g yeast extract certified (Difco.TM. 0127-01-7), 0.19 g MgSO.sub.4 anhydrous or 0.394 g MgSO.sub.4.7H.sub.2 O (Sigma.TM. M2773), 1.07 g **ammonium chloride** (Sigma.TM. A9434), 0.075 g KCl (Sigma.TM. P5405), 4.09 g NaCl (Sigma.TM. S3014), 120.0 mL of 1 M triethanolamine pH 7.4,. . .

DETD The supernatant was then passed over a **Protein** G-Sepharose.TM. Fast Flow (Pharmacia) column [0.5 mL bed volume] previously equilibrated by passing 10 mL TE buffer through the column.. . . added to concentrated eluant, and the resulting mixture was re-concentrated to 0.5 mL. SDS-PAGE gels were run to ascertain that **protein** had been produced.

DETD . . . on a reverse- phase PLRP-S.TM. 4.6.times.50 mm column, 8-mm particle size (Polymer Laboratories, Shropshire, UK), maintained at 50.degree. C. The **proteins** were eluted using an increasing

linear gradient from 31% B to 41% B. Buffer A contained 0.1% trifluoroacetic acid in. . . and Buffer B contained 0.1% trifluoroacetic acid in HPLC-grade acetonitrile. The flow rate was maintained at 2 mL/min, and the **protein** profile was monitored at 214 nm.

DETD . . . carried out on a Bakerbond carboxy-sulfon (CSX).TM. 50.times.4.6 mm column (J. T. Baker Phillipsburg, N.J.), maintained at 55.degree. C. The **proteins** were eluted using an increasing linear gradient from pH 6.0 to pH 8.0 at a flow rate of 2 mL/min. . .

DETD SDS-PAGE analysis was carried out on precast Novex.TM. gels (Novex, San Diego, Calif.). The **proteins** were stained using the Morrissey silver stain method. Morrissey, Anal. Biochem., 117: 307-310 (1981).

DETD . . . test (Associates of Cape Cod Inc., Woods Hole, Mass.). Samples containing less than 2 endotoxin units (Eu) per mg of **protein** were used in the pharmacokinetic studies.

DETD . . . 3.5. To this solution, pepsin (1 mg/mL) dissolved in 100 mM sodium citrate buffer, pH 3.5, was added at a pepsin-to-**protein** ratio of 1:12. After 4 hours at room temperature, the mixture's pH was raised to pH 6.4 with 10% NaOH.

DETD . . . described above for the Fab antibody fragment variants. After endotoxin determinations, samples containing less than 2 Eu per mg of **protein** were used in the pharmacokinetic studies set forth below.

L15 ANSWER 12 OF 109 USPATFULL

AB This invention is directed to the pharmaceutical use of phenyl compounds, which are linked to an aryl moiety by various linkages, for inhibiting tumor necrosis factor. The invention is also directed to the compounds, their preparation and pharmaceutical compositions containing these compounds. Furthermore, this invention is directed to the pharmaceutical use of the compounds for inhibiting cyclic AMP phosphodiesterase.

AN 2000:98453 USPATFULL

TI Compounds containing phenyl linked to aryl or heteroaryl by an aliphatic- or heteroatom-containing linking group

IN Ashton, Michael John, Dagenham, United Kingdom
Cook, David Charles, Dagenham, United Kingdom
Fenton, Garry, Dagenham, United Kingdom
Hills, Susan Jacqueline, Dagenham, United Kingdom
McFarlane, Ian Michael, Dagenham, United Kingdom
Morley, Andrew David, Dagenham, United Kingdom
Palfreyman, Malcolm Norman, Dagenham, United Kingdom
Ratcliffe, Andrew James, Dagenham, United Kingdom
Sharp, Brian William, Dagenham, United Kingdom
Thurairatnam, Sukanthini, Dagenham, United Kingdom
Vacher, Bernard Yvon Jack, Dagenham, United Kingdom
Vicker, Nigel, Dagenham, United Kingdom

PA Rhone-Poulenc Rorer Limited, West Malling, United Kingdom (non-U.S. corporation)

PI US 6096768 20000801 <--

AI US 1999-301877 19990429 (9)

RLI Continuation of Ser. No. US 1993-98178, filed on 28 Jul 1993, now patented, Pat. No. US 5935978 which is a continuation-in-part of Ser. No. WO 1992-GB153, filed on 28 Jan 1992, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Rotman, Alan L.; Assistant Examiner: Desai, Rita

LREP Oehler, Ross J., Newman, Irving

CLMN Number of Claims: 6

ECL Exemplary Claim: 1

DRWN No Drawings
LN.CNT 4735
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6096768 20000801 <--
SUMM . . . compounds, their preparation, pharmaceutical compositions containing these compounds, and their pharmaceutical use in the treatment of disease states associated with **proteins** that mediate cellular activity.
SUMM The principal in vivo actions of TNF can be broadly classified as inflammatory and **catabolic**. It has been implicated as a mediator of endotoxic shock, inflammation of joints and of the airways, immune deficiency states,. . .
SUMM . . . the anti-coagulant activity of vascular endothelial cells. The cachexia associated with certain disease states is mediated through indirect effects on **protein** catabolism. TNF also promotes bone resorption and acute phase **protein** synthesis.
DETD . . . hour, allowed to warm to room temperature and left to stand overnight. The mixture is then quenched with 10% aqueous **ammonium chloride** solution (150 mL), the layers separated and the aqueous layer further extracted with ethyl acetate (2.times.100 mL). The combined organic. . .
DETD . . . and then it is stirred for a further 6 hours. It is then treated with a saturated aqueous solution of **ammonium chloride** (300 mL), and concentrated in vacuo to low volume. The aqueous residue is extracted with ethyl acetate (2.times.200 mL). The. . .
DETD . . . for a further 2 hours in the cold, the mixture is filtered, and the filtrate is washed with saturated aqueous **ammonium chloride** solution. The organic phase is dried over sodium sulfate and evaporated. The resulting residue is subjected to flash chromatography, eluting. . .
DETD . . . to room temperature and the solution is stirred for a further 2 hours. The reaction mixture is treated with aqueous **ammonium chloride** solution (50 mL) and the solution is extracted with diethyl ether (2.times.200 mL). The combined extracts are dried and concentrated,. . .
DETD . . . 15 minutes, and stirred for a further 1 hour 30 minutes at -75.degree. C. The solution is treated with aqueous **ammonium chloride** solution and extracted with ethyl acetate (3.times.100 ml,). The organic layers are combined, washed with brine, dried and concentrated to. . .
DETD . . . 30 minutes. The resulting mixture is then allowed to warm to room temperature overnight, and then treated with saturated aqueous **ammonium chloride** solution (200 mL). The layers are separated and the aqueous layer is further extracted with ethyl acetate (3.times.300 mL). The. . .
DETD . . . is trimmed off and the endothelial layer on the intimal surface is removed by rubbing with a cotton swab. Smooth **muscle** strips are plucked from the aorta and 25 g are homogenized using a Waring Blender in homogenization buffer (20 mM. . .
DETD 3. Effects of Compounds on Tracheal Smooth **Muscle** Contractility.

L15 ANSWER 13 OF 109 USPATFULL
AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for

employing the disclosed compounds and compositions for treating
patients
requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 2000:95120 USPATFULL

TI Process for preparing steroid receptor modulator compounds

IN Jones, Todd K., Solana Beach, CA, United States
Goldman, Mark E., San Diego, CA, United States
Pooley, Charlotte L. F., San Diego, CA, United States
Winn, David T., San Diego, CA, United States
Edwards, James P., San Diego, CA, United States
West, Sarah J., San Diego, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)

PI US 6093821 20000725 <--

AI US 1997-943853 19971008 (8)

RLI Division of Ser. No. US 1995-464541, filed on 5 Jun 1995, now patented, Pat. No. US 5688810 which is a continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Huang, Evelyn Mei

LREP Elmer, J. Scott, Respess, William L.

CLMN Number of Claims: 12

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 11155

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6093821 20000725 <--

SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .

SUMM . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central enrvous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . . and cirrhosis. Accordingly, GR and MR active compounds have been
used
as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerabated herpes simplex virus.

SUMM . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.

DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated

ammonium chloride (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .

DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .

DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .

DETD . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .

DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . for the transcription-modulating activity of the target IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g, [.sup.3. . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

DETD . . . concentration (nM), requivuired to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.

DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . .

L15 ANSWER 14 OF 109 USPATFULL

AB The present invention relates to novel organic compounds, to methods for their preparation, to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis.

The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are compounds of formula (I) ##STR1## wherein (L).sub.n, Ar.sub.1; R.sub.1 and A are as defined in the application.

AN   2000:80778 USPATFULL
TI   Modulators of molecules with phosphotyrosine recognition units
IN   Andersen, Henrik Sune, Kobenhavn O, Denmark
        Moller, Niels Peter Hundahl, Kobenhavn O, Denmark
        Madsen, Peter, Bagsvaerd, Denmark
PA   Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI   US 6080770   20000627   <--
AI   US 1999-253419   19990219 (9)
RLI   Division of Ser. No. US 1997-842801, filed on 16 Apr 1997
PRAI   DK 1996-464   19960419
        US 1996-22116P   19960717 (60)
DT   Utility
FS   Granted
EXNAM   Primary Examiner: Richter, Johann; Assistant Examiner: Oswecki, Jane C.
LREP   Zelson, Steve T., Rozek, Carol E.
CLMN   Number of Claims: 9
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 2055
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 6080770   20000627   <--

AB . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are. . .

SUMM . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings.

SUMM Phosphorylation of **proteins** is a fundamental mechanism for regulation of many cellul processes. Although **protein** phosphorylation at serine and threonine residues quantitatively dominating in eukaryotic cells, reversible tyrosine phosphorylation seems play a pivotal role in. . .

SUMM The regulation of **protein** tyrosine phosphorylation in vivo is mediated by the opposing actions of **protein** tyrosine kinases (PTKs) and **protein** tyrosine phosphatases (PTPases). The level of **protein** tyrosine phosphorylation of cellular **proteins** is determined by the balanced activities of PTKs and PTPase (Hunter, 1995, supra).

SUMM The **protein** phosphatases are composed of at least two separate and distinct families (Hunter, T., Cell 58: 1013-1016 (1989)) the **protein** serine/threonine phosphatases and the PTPases.

SUMM Low molecular weight phosphotyrosine-**protein** phosphatase (LMW-PTPase) shows very little sequence identity to the intracellular

PTPases described above. However, this enzyme belongs to the PTPase. . .

SUMM . . . more than 500 different species will be found in the human genome, i.e. close to the predicted size of the **protein** tyrosine kinase superfamily (Hanks and Hunter, FASEB J. 9: 576-596 (1995)).

SUMM PTPases are the biological counterparts to **protein** tyrosine kinases (PTKs). Therefore, one important function of PTPases is to control, down-regulate, the activity of PTKs. However, a more. . .

SUMM Dual specificity **protein** tyrosine phosphatases (dsPTPases) define a subclass within the PTPases family that can hydrolyze phosphate from phosphortyrosine as well as from. . . His-Cys-Xxx-Xxx-Gly-Xxx-Xxx-Arg (SEQ ID NO: 2). At least three dsPTPases have been shown to dephosphorylate and inactivate extracellular signal-regulated kinase (ERKs)/mitogen-activated **protein** kinase (MAPK): MAPK phosphatase (CL100, 3CH134) (Charles et al., Proc. Natl. Acad. Sci. USA 90: 5292-5296 (1993)); PAC-1 (Ward et. . .

SUMM . . . domains and PTB domains primarily act as docking molecules with little or no catalytic activity. In other words, tyrosine phosphorylated **proteins** have the capacity to bind other **proteins** containing SH2 domains or PTB domains thereby controlling the subcellular location of signalling molecules. There appears to be a significant. . .

SUMM In an early study, vanadate was found to inhibit **protein** -tyrosine phosphatases in mammalian cells with a concomitant increase in the level of phosphotyrosine in cellular **proteins** leading to transformation (Karlund, Cell 41: 707-717 (1985)). Vanadium-based phosphatase inhibitors are relatively unspecific. Therefore, to assess the importance of. . .

SUMM . . . Mooney and Anderson, J. Biol. Chem. 264: 6850-6857 (1989)), with the tri-phosphorylated tyrosine-1150 domain being the most sensitive target for **protein**-tyrosine phosphatases (PTPases) as compared to the di- and mono- phosphorylated forms (King et al., Biochem. J. 275: 413-418 (1991)). It. . .

SUMM . . . be obtained in adipocytes (Fantus et al., Biochemistry 28: 8864-8871 (1989); Eriksson et al., Diabetologia 39: 235-242 (1995)) and skeletal **muscle** (Leighton et al., Biochem. J. 276: 289-292 (1991)). In addition, recent studies show that a new class of peroxovanadium compounds. . .

SUMM . . . signalling in a rat hepatoma cell line (Kulas et al., J. Biol. Chem. 270: 2435-2438 (1995)). A suppression of LAR **protein** levels by about 60 percent was paralleled by an approximately 150 percent increase in insulin-induced auto-phosphorylation. However, only a modest. . .

SUMM . . . the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family **protein** -tyrosine kinase (Mustelin et al., Proc. Natl. Acad. Sci. USA 86: 6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. USA. . . to T-cell activation. In a recent study it was found that recombinant p56.sup.lck specifically associates with recombinant CD45 cytoplasmic domain **protein**, but not to the cytoplasmic domain of the related PTP.alpha. (Ng et al., J. Biol. Chem. 271: 1295-1300 (1996)). The. . . mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosine. In immature B cells, another member of the Src family **protein**-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri et al.,

J. Biol.. . .

SUMM . . . fibroblasts grow on appropriate substrates, seem to mimic, at least in part cells and their natural surroundings. Several focal adhesion **proteins** are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, Neuron 11, 551-564 (1993)). However, aberrant tyrosine phosphorylation of these **proteins** can lead to cellular transformation. The intimate association between PTPases and focal adhesions is supported by the finding of several. . . PTPD1 (M.o slashed.ller et al., Proc. Natl. Acad. Sci. USA 91: 7477-7481 (1994)). The ezrin-like domain show similarity to several **proteins** that are believed to act as links between the cell membrane and the cytoskeleton. PTPD1 was found to be phosphorylated. . .

SUMM PTPases may oppose the action of tyrosine kinases, including those responsible for phosphorylation of focal adhesion **proteins**, and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool et. . .

SUMM . . . al., J. Biol. Chem. 260: 4653-4660 (1985); Lau et al., J. Biol. Chem. 262: 1389-1397 (1987); Lau et al., Adv. **Protein** Phosphatases 4: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when. . .

SUMM In preferred embodiments, the compounds of the invention modulate the activity of **protein** tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s).

SUMM In one preferred embodiment the compounds of the invention act as inhibitors of PTPases, e.g. **protein** tyrosine phosphatases involved in regulation of tyrosine kinase signalling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signalling pathways via. . .

SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis, stimulation of the immune system; treatment of retardation, acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein** **catabolic** responses after major surgery; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidio-blastosis; Adjuvant treatment for ovulation induction; stimulation of thymic development and prevention the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling and. . .

SUMM Phosphotyrosine recognition unit/tyrosine phosphate recognition units/pTyr recognition units are defined as areas or domains of **proteins** or glycoproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not. . .

SUMM PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing **proteins** or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the. . .

SUMM . . . recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of **proteins** or glycoproteins with pTyr recognition units (e.g. PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule wit a **protein** or glyco-**protein** with pTyr recognition units either via a direct action on the pTyr recognition site or via an indirect mechanism. Examples. . . leading to initiation of normal or abnormal cellular activity, e) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to initiation of normal or abnormal cellular activity.

DETD A mixture of the above acetonitrile (2.50 g, 15 mmol), **ammonium chloride** (1.60 g, 30 mmol) and sodium azide (1.94 g, 30 mmol) in N,N'-dimethylformamide (25 ml) was stirred at 125.degree. C.. . . .

DETD A mixture of the above acetonitrile (5.40 g, 32 mmol), **ammonium chloride** (2.59 g, 48 mmol) and sodium azide (3.15 g, 48 mmol) in N,N'-dimethylformamide (100 ml) was stirred at 125.degree. C.. . . .

DETD . . . full-length sequence of PTP1B and the intracellular part of PTP.alpha. were introduced into the insect cell expression vector pVL1392. The **proteins** were expressed according to standard procedures. PTP1B was semi-purified by ion exchange chromatography, and PTP.alpha. was purified to apparent homogeneity. . .

L15 ANSWER 15 OF 109 USPATFULL

AB A new class of xanthine compounds, variously substituted at the 1, 3, 7 and 8 positions, is characterized by an ability to modulate the activity of key enzymes involved in drug metabolism. These compounds generally are useful in affecting drug metabolism and, particularly, in extending the circulating half-life of compounds that are metabolized via P-450-mediated pathways.

AN 2000:74294 USPATFULL

TI Xanthine modulators of metabolism of cellular P-450

IN Klein, J. Peter, Vashon, WA, United States
Kumar, Anil M., Seattle, WA, United States
Woodson, Paul, Edmonds, WA, United States

PA Cell Therapeutics, Inc., Seattle, WA, United States (U.S. corporation)

PI US 6075029 20000613 <--

AI US 1998-2345 19980102 (9)

DT Utility

FS Granted

EXNAM Primary Examiner: Berch, Mark L.

LREP Foley & Lardner

CLMN Number of Claims: 14

ECL Exemplary Claim: 1,2

DRWN No Drawings

LN.CNT 808

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6075029 20000613 <--

SUMM . . . to the liver before it is passed to the rest of the body. On this first pass, high levels of **catabolic** enzymes in the liver results in significant metabolism of the drug before it can reach its intended site of action,. . .

DETD . . . 70-80.degree. C. for 5 hours. After evaporation of the solvent

under reduced pressure, the residue was suspended in saturated aqueous **ammonium chloride** solution (50 ml) and extracted with ethyl acetate (3.times.75 ml). The combined extracts were washed with saturated aqueous sodium chloride. . .

DETD To 200 .mu.L microsomal suspension (HL-124; 20 mg/mL **protein**, P-450 specific activity 0.24 nmol/mg **protein**) and 100 .mu.L 8.0 mM NADPH (Sigma, tetrasodium salt) in 100 mM phosphate buffer pH 7.4 preincubated for 2 minutes. . .

DETD . . . was added as an internal standard. Paraxanthine standards were prepared in 0.5 mL phosphate buffer containing 2.0 mg boiled microsomal **protein** ranging in concentration from 0.433 to 13.9 nmol/sample.

L15 ANSWER 16 OF 109 USPATFULL

AB A class of substituted and unsubstituted nucleo-base analogs and related azoles, designated as "phosphazoles," is disclosed, certain preferred embodiments having the basic structure of ##STR1## Also disclosed are methods of making and using the new compounds.

AN 2000:67721 USPATFULL

TI Phosphazole compounds

IN Revanker, Ganapathi R., 180 N. Milltrace Dr., The Woodlands, TX, United States 77381

PI US 6069132 20000530 <--

AI US 1997-910291 19970813 (8)

PRAI US 1996-23907P 19960814 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Crane, L. Eric

LREP McGregor, Martin L.

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 12 Drawing Page(s)

LN.CNT 2155

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6069132 20000530 <--

SUMM Tumor necrosis factor alpha (TNF.alpha.), a mononuclear phagocytic cell derived **protein**, was originally described as a product of activated macrophages and shown to display tumoricidal activity (6-9). Extensive research during the. . . cachexia, inflammation, autoimmunity, and other immunological and pathological reactions (11). There are two forms of TNF.alpha., a type II membrane **protein** of relative molecular mass 26,000 (26 kDa) and a soluble, 17 kDa form generated from the cell-bound **protein** by proteolytic cleavage. Several different types of tumors have been described in which TNF.alpha. acts as an autocrine growth factor,. . .

DETD . . . 86 is converted into the corresponding carboxamidine (compound 87, X.dbd.NH) and carboxamidoxime (compound 87, X.dbd.NOH) by the treatment with liquid anmmonia/**ammonium chloride** and hydroxylamine, respectively. The carbonitrile function of compound 86 is also available for further transformation reactions.

DETD Adenosine deaminase (ADA) is a ubiquitous **catabolic** enzyme present in many animal and human tissues (112). In addition to converting adenosine to inosine, this enzyme catalyzes the. . .

DETD . . . may include carbowax. Additionally, the compounds of the present invention are suitable for encapsulation in liposomes or for crosslinking with **protein** carriers and the like. The pharmaceutical compositions of the present invention may be administered

by conventional methods such as are. . .

L15 ANSWER 17 OF 109 USPATFULL

AB The present invention relates to novel organic compounds, to methods for their preparation, to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis.
The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are compounds of formula (I)

(L).sub.n --Ar.sub.1 --R.sub.1 --A (I)

wherein

(L).sub.n, n, Ar.sub.1, R.sub.1 and A are as defined in the application.

AN 2000:61616 USPATFULL
TI Modulators of molecules with phosphotyrosine recognition units
IN Andersen, Henrik Sune, Kobenhavn O, Denmark
Moller, Niels Peter Hundahl, Kobenhavn O, Denmark
Madsen, Peter, Bagsvaerd, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 6063800 20000516 <--
AI US 1999-253443 19990219 (9)
RLI Division of Ser. No. US 1997-842801, filed on 16 Apr 1997
PRAI DK 1996-464 19960419
US 1996-22116P 19960717 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Richter, Johann; Assistant Examiner: Oswecki, Jane C.
LREP Zelson, Steve T., Rozek, Carol E.
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2073
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6063800 20000516 <--
AB . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are. . .
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SUMM . . . signalling in a rat hepatoma cell line (Kulas et al., J. Biol. Chem. 270: 2435-2438 (1995)). A suspension of LAR **protein** levels by about 60 percent was paralleled by an approximately 150

percent increase in insulin-induced auto-phosphorylation. However, only a modest. . .

SUMM . . . the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family **protein** -tyrosine kinase (Mustelin et al., Proc. Natl. Acad. Sci. USA 86: 6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. USA. . . to T-cell activation. In a recent study it was found that recombinant p56.sup.lck specifically associates with recombinant CD45 cytoplasmic domain **protein**, but not to the cytoplasmic domain of the related PTP.alpha. (Ng et al., J. Biol. Chem. 271: 1295-1300 (1996)). The. . . mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosien. In immature B cells, another member of the Src family **protein**-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri et al., J. Biol.. . .

SUMM . . . fibroblasts grow on appropriate substrates, seem to mimic, at least in part, cells and their natural surroundings. Several focal adhesion **proteins** are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, Neuron 11, 551-564 (1993)). However, aberrant tyrosine phosphorylation of these **proteins** can lead to cellular transformation. The intimate association between PTPases and focal adhesions is supported by the finding of several. . . and PTPD1 (Moller et al., Proc. Natl. Acad. Sci. USA 91: 7477-7481 (1994)). The ezrin-like domain show similarity to several **proteins** that are believed to act as links between the cell membrane and the cytoskeleton. PTPD1 was found to be phosphorylated. . .

SUMM PTPases may oppose the action of tyrosine kinases, including those responsible for phosphorylation of focal adhesion **proteins**, and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool et. . .

SUMM . . . al., J. Biol. Chem. 260: 4653-4660 (1985); Lau et al., J. Biol. Chem. 262: 1389-1397 (1987); Lau et al., Adv. **Protein** Phosphatase 4: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when. . .

SUMM In preferred embodiments, the compounds of the invention modulate the activity of **protein** tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s).

SUMM In one preferred embodiment the compounds of the invention act as inhibitors of PTPases, e.g. **protein** tyrosine phosphatases involved in regulation of tyrosine kinase signalling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signalling pathways via. . .

SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocortidoids; treatment of osteoporosis, stimulation of the immune system; treatment of retardation, acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein** **catabolic** responses after major surgery; reducing cachexia and

protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidio-blastosis; Adjuvant treatment for ovulation induction; stimulation of thymic development and
prevention the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling and. . .

SUMM Phosphotyrosine recognition units/tyrosine phosphate recognition units/pTyr recognition units are defined as areas or domains of **proteins** or glycorproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not. . .

SUMM PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing **proteins** or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the. . .

SUMM . . . recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of **proteins** or glycoproteins with pTyr recognition units (e.g. PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule with a **protein** or glyco-**protein** with pTyr recognition units either via a direct action on the pTyr recognition site or via an indirect mechanism. Examples. . . leading to initiation of normal or abnormal cellular activity; e) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to initiation of normal or abnormal cellular activity.

DETD A mixture of the above acetonitrile (2.50 g, 15 mmol), **ammonium chloride** (1.60 g, 30 mmol) and sodium azide (1.94 g, 30 mmol) in N,N'-dimethylformamide (25 ml) was stirred at 125.degree. C.. . . .

DETD A mixture of the above acetonitrile (5.40 g, 32 mmol), **ammonium chloride** (2.59 g, 48 mmol) and sodium azide (3.15 g, 48 mmol) in N,N'-dimethylformamide (100 ml) was stirred at 125.degree. C.. . . .

DETD . . . full-length sequence of PTP1B and the intracellular part of PTP.alpha. were introduced into the insect cell expression vector pVL1392. The **proteins** were expressed according to standard procedures. PTP1B was semi-purified by ion exchange chromatography, and PTP.alpha. was purified to apparent homogeneity. . .

L15 ANSWER 18 OF 109 USPATFULL

AB This invention is directed to compounds of formula I: ##STR1## wherein the variables are as described herein. Compounds within the scope of the
present invention possess useful properties, more particularly pharmaceutical properties. They are especially useful for inhibiting the
production or physiological effects of TNF in the treatment of a patient
suffering from a disease state associated with a physiologically detrimental excess of tumor necrosis factor (TNF). Compounds within the scope of the present invention also inhibit cyclic AMP phosphodiesterase, and are useful in treating a disease state associated
with pathological conditions that are modulated by inhibiting cyclic AMP
phosphodiesterase, such disease states including inflammatory and

autoimmune diseases, in particular type IV cyclic AMP
phosphodiesterase.
Compounds within the scope of the present invention may also inhibit an MMP, and are useful in treating a disease state associated with pathological conditions that are modulated by inhibiting MMPs, such disease states involve tissue breakdown and those associated with a physiologically detrimental excess of TNF. The present invention is therefore also directed to the pharmaceutical use of the compounds, pharmaceutical compositions containing the compounds, intermediates leading thereto and methods for the preparation of the compounds and their intermediates.

AN 2000:54152 USPATFULL
TI Substituted (aryl, heteroaryl, arylmethyl or heteroarylmethyl) hydroxamic acid compounds
IN Groneberg, Robert D., Collegeville, PA, United States
Neuenschwander, Kent W., Schwensville, PA, United States
Djuric, Stevan W., Libertyville, IL, United States
McGeehan, Gerald M., Chester Springs, PA, United States
Burns, Christopher J., Rosemont, PA, United States
Condon, Steven M., Chester Springs, PA, United States
Morrissette, Matthew M., Pottstown, PA, United States
Salvino, Joseph M., Schwenksville, PA, United States
Scotese, Anthony C., King of Prussia, PA, United States
Ullrich, John W., Schwenksville, PA, United States
PA Rhone-Poulenc Rorer Pharmaceuticals Inc., Collegeville, PA, United States (U.S. corporation)
PI US 6057369 20000502 <--
AI US 1997-928943 19970912 (8)
RLI Continuation of Ser. No. WO 1997-US264, filed on 2 Jan 1997
DT Utility
FS Granted
EXNAM Primary Examiner: Burn, Brian M.; Assistant Examiner: Davis, Brian J.
LREP Parker, III, Raymond S.
CLMN Number of Claims: 93
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 7035
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6057369 20000502 <--
SUMM . . . compounds, their preparation, pharmaceutical compositions containing these compounds, and their pharmaceutical use in the treatment of disease states associated with **proteins** that mediate cellular activity which are capable of being modulated by inhibiting a matrix metalloproteinase (MMP), tumor necrosis factor
(TNF)
or cyclic AMP phosphodiesterase, or **proteins** associated therewith that mediate cellular activity. This invention is also directed to intermediates useful in preparing the (aryl, heteroaryl, aralkyl. . .
SUMM The principal in vivo actions of TNF can be broadly classified as inflammatory and **catabolic**. It has been implicated as a mediator of endotoxic shock, inflammation of joints and of the airways, immune deficiency states,. . .
SUMM . . . the anti-coagulant activity of vascular endothelial cells. The cachexia associated with certain disease states is mediated through indirect effects on **protein** catabolism. TNF also promotes bone resorption and acute phase **protein** synthesis.
DETD . . . dropwise over 3 minutes. The mixture is heated under reflux
for
3 days. The mixture is added to a saturated **ammonium**

chloride solution and is extracted with ether. The organic layer
is washed with water and is dried over MgSO.sub.4. The solvent. . .

DETD . . . and the resulting pale yellow, heterogeneous mixture is stirred
at 0.degree. C. for 30 minutes then quenched with saturated aqueous
ammonium chloride solution.

DETD . . . ester enolate dropwise over 35 minutes. This mixture is stirred
for 1 hour at -78.degree. C. and then quenched with **ammonium**
chloride solution (25 mL). Additional water (100 mL) is added to
break up the emulsion that has formed. The layers are. . .

DETD . . . and subsequently washed with 2% sodium dodecyl sulphate in
phosphate buffered saline followed by phosphate buffered saline to
remove contaminating **proteins** and nucleic acids. The cell wall
is further purified by sonication and differential centrifugation to
obtain a purified preparation which. . .

L15 ANSWER 19 OF 109 USPATFULL

AB The present application describes novel lactams and derivatives thereof
of formula I: ##STR1## or pharmaceutically acceptable salt forms
thereof, wherein rings ring B is a 4-8 membered cyclic amide containing
from 0-3 additional heteroatoms selected from N, O, and S, which are
useful as metalloprotease inhibitors.

AN 2000:54119 USPATFULL

TI Lactam metalloprotease inhibitors

IN Duan, Jingwu, Newark, DE, United States
Decicco, Carl P., Newark, DE, United States
Wasserman, Zelda R., Wilmington, DE, United States
Maduskuie, Jr., Thomas P., Wilmington, DE, United States

PA E. I. du Pont de Nemours and Company, Wilmington, DE, United States
(U.S. corporation)

PI US 6057336 20000502 <--

AI US 1998-165747 19981002 (9)

PRAI US 1997-62418P 19971003 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Richter, Johann; Assistant Examiner: Keating, Dominic

LREP Vance, David H.

CLMN Number of Claims: 54

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 8712

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6057336 20000502 <--

SUMM . . . as rheumatoid and osteoarthritis, corneal, epidermal or gastric
ulceration; tumor metastasis or invasion; periodontal disease and bone
disease. Normally these **catabolic** enzymes are tightly
regulated at the level of their synthesis as well as at their level of
extracellular activity through. . .

SUMM . . . of cartilage degradation in OA (Mankin et al. J. Bone Joint
Surg. 52A, 1970, 424-434). There are four classes of **protein**
degradative enzymes in mammalian cells: serine, cysteine, aspartic and
metalloproteinases. The available evidence supports that it is the
metalloproteinases which. . .

SUMM . . . as amino acids which are known to occur biologically in free or
combined form but usually do not occur in **proteins**. Included
within this term are modified and unusual amino acids,such as those
disclosed in, for example, Roberts and Vellaccio (1983) The Peptides,

342-429, the teaching of which is hereby incorporated by reference. Natural **protein** occurring amino acids include, but are not limited to, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, tyrosine, tryptophan, proline, and valine. Natural non-**protein** amino acids include, but are not limited to arginosuccinic acid, citrulline, cysteine sulfinic acid, 3,4-dihydroxyphenylalanine, homocysteine, homoserine, ornithine, 3-monoiodotyrosine, 3,5-diiodotryosine,. . .

DETD . . . After 1 h at -78.degree. C., iodomethane (18.2 mL, 1.5 eq) was added. After 2 h at -20.degree. C., saturated **ammonium chloride** (400 mL), water (600 mL), ether (500 mL) and hexane (500 mL) were added. The two phases were separated and. . .

DETD . . . C., the cold bath was removed and the mixture stirred at ambient temperature for 1 h. Following addition of saturated **ammonium chloride** (200 mL), water (800 mL), and hexane (1000 mL), the two phases were separated and the aqueous phase extracted with. . .

DETD . . . 0.383 mmol), and p-t-butylbenzyl bromide (174 mg, 2 eq) in methyl sulfoxide (2 mL). After 1.5 h at rt, saturated **ammonium chloride** (3 mL) and ethyl acetate (100 mL) were added. The mixture was washed with water (2.times.5 mL), brine (5 mL),. . .

DETD . . . h at rt, same portions of cesium carbonate and 2-picolyl chloride were added. After 1 h at 50.degree. C., saturated **ammonium chloride** (6 mL) and ethyl acetate (100 mL) were added. The mixture was washed with water (6 mL), brine (6 mL),. . .

DETD . . . at rt, same portions of cesium carbonate and 3-picolyl chloride hydrochloride were added. After 2 h at 75.degree. C., saturated **ammonium chloride** (6 mL) and ethyl acetate (100 mL) were added. The mixture was washed with water (6 mL), brine (6 mL),. . .

DETD . . . at rt, same portions of cesium carbonate and 4-picolyl chloride hydrochloride were added. After 30 min at 75.degree. C., saturated **ammonium chloride** (6 mL) and ethyl acetate (100 mL) were added. The mixture was washed with water (6 mL), brine (6 mL),. . .

DETD . . . bromide (1.71 mL, 1.05 eq) in tetrahydrofuran (25 mL) was added dropwise. After additional 2 h at 0.degree. C., saturated **ammonium chloride** (50 mL) was added and the mixture extracted with ethyl acetate (3.times.). The combined extracts were washed with brine, dried. . .

DETD . . . at 0.degree. C. the mixture was stirred at rt for 24 h and at 60.degree. C. for 3 h. Sat **ammonium chloride** was added and the mixture extracted with ethyl acetate (2.times.). The extracts were washed with sat NaHCO3, water and brine,. . .

DETD . . . (120 mg, 0.433 mmol) and 1-fluoro-4-nitrobenzene (122 mg, 2 eq) in DMSO (2 mL). After 1 h at rt, sat **ammonium chloride** (3 mL) and ethyl acetate (100 mL) were added. The mixture was washed with water (2.times.5 mL), brine (5 mL),. . .

DETD . . . (120 mg, 0.339 mmol) and 2,6-dimethyl-4-phenol (83 mg, 2 eq) in DMSO (4 mL). After 3 h at rt, sat **ammonium chloride** was added. The mixture was extracted with ethyl acetate (3.times.). The

combined extracts were washed with brine, dried (MgSO4) and. . .
DETD . . . (12.0 mL, 2 eq) and di-t-butyl dicarbonate (8.33 g, 1.2 eq) for
1 h at rt. Following addition of sat **ammonium chloride** (50 mL) and ethyl acetate (800 mL), the mixture was washed with water (2.times.50 mL), brine (50 mL), dried (MgSO4). . .
DETD . . . in DMF (10 mL) at rt. The reaction was stirred for 48 h, diluted with ethyl acetate, washed with saturated **ammonium chloride**, dried over magnesium sulfate and concentrated to give an oil. The crude was purified by chromatography on silica gel eluting. . .
DETD . . . bromide (3.53 mL, 3 eq) in DMSO at rt. After 1 h at this temperature, ether (800 mL) and sat **ammonium chloride** (100 mL) were added. The organic phase was separated, washed with water (3.times.50 mL), brine (50 mL), dried (MgSO4) and. . .
DETD . . . 2-benzyloxyethyl iodide (50.45 g, 1.1 eq) in THF (40 mL) was added dropwise. After 2 h at 0.degree. C., sat **ammonium chloride** (500 mL) was added. Following removal of THF in vacuo, the residue was diluted with water (250 mL) and extracted. . .
DETD . . . is detected by monitoring production of aggrecan fragments produced exclusively by cleavage at the Glu373-Ala374 bond within the aggrecan core **protein** by Western analysis using the monoclonal antibody, BC-3 (Hughes, CE, et al., Biochem J 306:799-804, 1995). This antibody recognizes aggrecan. . . when it is at the N-terminus and not when it is present internally within aggrecan fragments or within the aggrecan **protein** core. Other proteases produced by cartilage in response to IL-1 do not cleave aggrecan at the Glu373-Ala374 aggrecanase site; therefore,. . .
DETD . . . the glycosaminoglycan side chains from aggrecan is necessary for the BC-3 antibody to recognize the ARGSVIL epitope on the core **protein**. Therefore, for analysis of aggrecan fragments generated by cleavage at the Glu373-Ala374 site, proteoglycans and proteoglycan fragments are enzymatically deglycosylated. . .

L15 ANSWER 20 OF 109 USPATFULL
AB Methods of using an aqueous neutral to mildly alkaline metal bicarbonate solution are disclosed. The solution comprises metal bicarbonate dissolved in the solution, the metal bicarbonate comprising bicarbonate anions and metal cations. In addition there is a pH adjusting agent in the solution in an amount whereby the solution is at a neutral to mildly alkaline pH. The disclosed methods are for preventing or for treating inflammatory diseases or degenerative diseases in a mammal and for preventing or treating viral diseases in a mammal.
AN 2000:43794 USPATFULL
TI Aqueous metal bicarbonate solution useful in treating inflammatory, degenerative and viral diseases
IN Beckett, Russell John, Red Hill, Australia
PA Macquarie Veterinary Supplies Pty Ltd, Red Hill, Australia (non-U.S. corporation)
PI US 6048553 20000411 <--
AI US 1998-41787 19980313 (9)
PRAI AU 1997-5677 19970317
AU 1997-608 19971128
DT Utility
FS Granted
EXNAM Primary Examiner: Jarvis, William R. A.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1

DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 2772
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6048553 20000411 <--

SUMM Typically the certain viral diseases require intracellular acidic conditions or intracellular proton concentrations for either removal of viral **protein** coats or assembly of viral **protein** coats. Typically the viral diseases may present as influenza.

SUMM Typically the certain viral diseases require intracellular acidic conditions or intracellular proton concentrations for either removal of viral **protein** coats or assembly of viral **protein** coats. Typically the viral diseases may present as influenza.

SUMM . . . of one month to the maximum consumption. This start-up schedule generally avoids any gastrointestinal side effects due to the smooth **muscle** relaxation properties of magnesium.

DETD . . . of one month to the maximum consumption. This start-up schedule generally avoids any gastrointestinal side effects due to the smooth **muscle** relaxation properties of magnesium.

DETD . . . made using a trapped fluorescein derivative. An increase in intracellular proton concentrations (intracellular acidification) was achieved by applying 10 mmol **ammonium chloride** (NH.sub.4 Cl) solution to a suspension of cells and then removing the NH.sub.4 Cl. An increase in intracellular bicarbonate concentrations. . .

DETD B. 10 mmol **ammonium chloride** (NH.sub.4 Cl) solution pH 7.5 applied to suspension of leucocytes for 10 minutes.

DETD . . . with chronic inflammation, the diseases associated with lysosomal enzyme activities, the diseases associated with oxidations of cell nucleic acids, cell **protein** amino acids and cell membrane lipids, and the diseases associated with aberrations of mitochondrial respiration.

DETD . . . oxidations of the structural and function molecules that constitute body cells and tissues. These oxidations occur particularly in nucleic acids, **protein** amino acids and cell membrane lipids.

DETD Oxidations of nucleic acids and **protein** amino acids lead to nucleic acid and **protein** degradation respectively. These degradations lead to senescence in mammals. Nucleic acid degradation is manifested by either cell death or cell transformation to the cancerous state. **Protein** degradation is manifested by increased urea concentrations in the body which can be detected in the plasma.

DETD Determination of plasma urea concentrations in elderly mammals is a direct measure of amino acid oxidation, **protein** degradation and overall nitrogen (anabolic/**catabolic**) balance. Determination of plasma urea concentrations in elderly mammals is a direct measure of cellular degenerations and senescence.

DETD The consumption of aqueous metal bicarbonate solution, principally magnesium bicarbonate solution, decreases amino acid oxidations, decreases **protein** degradation and improves overall nitrogen (anabolic/**catabolic**) balance in mammals. The consumption of aqueous metal bicarbonate solution, principally magnesium bicarbonate solution, delays cellular degenerations and senescence in. . .

DETD Increased proton concentrations from the hydrolysis of ATP occur particularly in the cytoplasm of **muscle** cells during muscular (motor) activity. This is referred to often as an increase in `lactic acid` (the lactic acid is,. . .

DETD . . . cell processes and cell functions. Mitochondrial inefficiency

arises from oxidative damage to mitochondrial nucleic acids, mitochondrial enzymes and mitochondrial membrane **proteins** and lipids, Inefficient mitochondria gradually and progressively dominate in body cells through middle age to old age. Middle aged and. . .

DETD . . . acid-dependent enzymes which utilise proton concentrations in host cells. For example, the acid proteases of lentiviruses are required for virus **protein** assembly and viral infectivity.

DETD . . . the bronchial mucosa. It is complicated often by bacterial pneumonia. Clinical signs of influenza include initially fever, malaise, headache and **muscle** pain followed by coughing, sneezing and respiratory tract effusions. Flu-like respiratory viruses cause respiratory diseases manifested generally by clinical signs. . .

DETD . . . to the consequent increase in hunger it produces, the large increase in carbon dioxide concentrations that occur with increased aerobic **muscle** activity and the damage excess activity does to inefficient mitochondria. Indeed, active **muscle** cells contain mitochondria with most nucleic acid damage relative to other body cells.

DETD . . . period of one month to the maximum consumption. This start-up schedule avoided any gastrointestinal side effects due to the smooth **muscle** relaxation properties of magnesium. Capillary dilation in the face was apparent in several people (which was interpreted by those affected. . .

DETD A heart **muscle** cell contains mitochondria that occupy one quarter of the cell volume. It is natural to expect the heart to be. . . heart and its subsequent requirement for `energy`. The consumption of magnesium bicarbonate may assist in maintaining efficient mitochondria in heart **muscle** cells. In the presence of bicarbonate anions, mitochondrial efficiency in heart **muscle** cells is maintained by processes which include decreases in proton leaks across inner mitochondrial membranes, establishment of proton circuits independent. . . in mitochondrial matrixes. In the presence of bicarbonate anions, mitochondrial damage and mitochondrial failure are decreased. Efficient mitochondria in heart **muscle** cells maintain ATP production so that the heart remains functional as a vital organ.

DETD . . . effects were observed in people participating in the trial reported in Example 5. Second, lysosomal enzyme damage to ischaemic heart **muscle** may be prevented or decreased.

DETD . . . the damage to the complex molecules of the mitochondria. For example, the mitochondria of aged people carry nucleic acid and **protein** defects not observed in the mitochondria of young people. This is true particularly of the mitochondria in **muscle**, heart and brain cells. Accordingly, it has been proposed that several chronic diseases common in old age may be related. . .

L15 ANSWER 21 OF 109 USPATFULL

AB The present invention relates to novel substituted acrylic acids, to methods for their preparation, compositions containing them, and their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention also relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings.

AN 2000:37803 USPATFULL

TI Modulators of molecules with phosphotyrosine recognition units
IN Andersen, Henrik Sune, K.o slashed.benhavn, Denmark
M.o slashed.ller, Niels Peter Hundahl, K.o slashed.benhavn, Denmark
Madsen, Peter, Bagsv.ae butted.rd, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 6043247 20000328 <--
AI US 1997-842800 19970416 (8)
PRAI DK 1996-463 19960419
DK 1996-1436 19961217
US 1996-23661P 19960717 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Covington, Raymond
LREP Zelson, Esq., Steve T., Lambiris, Esq., Elias
CLMN Number of Claims: 35
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1777
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6043247 20000328 <--
AB . . . preparation, compositions containing them, and their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis.
The invention also relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings.

SUMM . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings.

SUMM Phosphorylation of **proteins** is a fundamental mechanism for regulation of many cellular processes. Although **protein** phosphorylation at serine and threonine residues is quantitatively dominating in eukaryotic cells, reversible tyrosine phosphorylation seems to play a pivotal. . . .

SUMM The regulation of **protein** tyrosine phosphorylation in vivo is mediated by the opposing actions of **protein** tyrosine kinases (PTKs) and **protein** tyrosine phosphatases (PTPases). The level of **protein** tyrosine phosphorylation of cellular **proteins** is determined by the balanced activities of PTKs and PTPase (Hunter, 1995, supra).

SUMM The **protein** phosphatases are composed of at least two separate and distinct families (Hunter, T., Cell 58: 1013-1016 (1989)) the **protein** serine/threonine phosphatases and PTPases.

SUMM Low molecular weight phosphotyrosine-**protein** phosphatase (LMW-PTPase) shows very little sequence identity to the intracellular PTPases described above. However, this enzyme belongs to the PTPase. . .

SUMM . . . more than 500 different species will be found in the human genome, i.e. close to the predicted size of the **protein** tyrosine kinase superfamily (Hanks and Hunter, FASEB J. 9: 576-596 (1995)).

SUMM PTPases are the biological counterparts to **protein** tyrosine kinases (PTKs). Therefore, one important function of PTPases is to

control, down-regulate, the activity of PTKs. However, a more. . .
SUMM Dual specificity **protein** tyrosine phosphatases (dsPTPases) define a subclass within the PTPases family that can hydrolyze phosphate from phosphortyrosine as well as from. . . PTPases: His-Cys-Xxx-Xxx-Gly-Xxx-Xxx-Arg (SEQ ID NO:2). At least three dsPTPases have been shown to dephosphorylate and inactivate extracellular signal-regulated kinase (ERKs)/mitogen-activated **protein** kinase (MAPK): MAPK phosphatase (CL100, 3CH134) (Charles et al., Proc. Natl. Acad. Sci. U.S.A. 90: 5292-5296 (1993)); PAC-1 (Ward et. . .
SUMM . . . domains and PTB domains primarily act as docking molecules with little or no catalytic activity. In other words, tyrosine phosphorylated **proteins** have the capacity to bind other **proteins** containing SH2 domains or PTB domains thereby controlling the subcellular location of signaling molecules. There appears to be a significant. . .
SUMM In an early study, vanadate was found to inhibit **protein** -tyrosine phosphatases in mammalian cells with a concomitant increase in the level of phosphotyrosine in cellular **proteins** leading to transformation (Klarlund, Cell 41: 707-717 (1985)). Vanadium-based phosphatase inhibitors are relatively unspecific. Therefore, to assess the importance of. . .
SUMM . . . Mooney and Anderson, J. Biol. Chem. 264: 6850-6857 (1989)), with the tri-phosphorylated tyrosine-1150 domain being the most sensitive target for **protein**-tyrosine phosphatases (PTPases) as compared to the di- and mono phosphorylated forms (King et al., Biochem. J. 275: 413-418 (1991)). It. . .
SUMM . . . be obtained in adipocytes (Fantus et al., Biochemistry 28: 8864-8871 (1989); Eriksson et al., Diabetologia 39: 235-242 (1995)) and skeletal **muscle** (Leighton et al., Biochem. J. 276: 289-292 (1991)). In addition, recent studies show that a new class of peroxovanadium compounds. . .
SUMM . . . signaling in a rat hepatoma cell line (Kulas et al., J. Biol. Chem. 270: 2435-2438 (1995)). A suppression of LAR **protein** levels by about 60 percent was paralleled by an approximately 150 percent increase in insulin-induced auto-phosphorylation. However, only a modest. . .
SUMM . . . the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family **protein** -tyrosine kinase (Mustelin et al., Proc. Natl. Acad. Sci. U.S.A. 86: 6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. U.S.A.. . . to T-cell activation. In a recent study it was found that recombinant p56.sup.lck specifically associates with recombinant CD45 cytoplasmic domain **protein**, but not to the cytoplasmic domain of the related PTP.alpha. (Ng et al., J. Biol. Chem. 271: 1295-1300 (1996)). The. . . mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosine. In immature B cells, another member of the Src family **protein**-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri et al., J. Biol.. . .
SUMM . . . fibroblasts grow on appropriate substrates, seem to mimic, at least in part, cells and their natural surroundings. Several focal adhesion **proteins** are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, Neuron 11, 551-564 (1993)). However, aberrant tyrosine phosphorylation of these **proteins** can lead to cellular transformation. The

intimate association between PTPases and focal adhesions is supported
by
the finding of several. . . and PTPD1 (M.o slashed.ller et al.,
Proc.
Natl. Acad. Sci. U.S.A. 91: 7477-7481(1994)). The ezrin-like domain
show
similarity to several **proteins** that are believed to act as
links between the cell membrane and the cytoskeleton. PTPD1 was found
to
be phosphorylated. . .

SUMM PTPases may oppose the action of tyrosine kinases, including those responsible for phosphorylation of focal adhesion **proteins**, and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool et. . .

SUMM . . . al., J. Biol. Chem. 260: 4653-4660 (1985); Lau et al., J.
Biol.
Chem. 262: 1389-1397 (1987); Lau et al., Adv. **Protein** Phosphatases 4: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when. . .

SUMM In preferred embodiments, the compounds of the invention modulate the activity of **protein** tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s).

SUMM In one preferred embodiment the compounds of the invention act as inhibitors of PTPases, e.g. **protein** tyrosine phosphatases involved in regulation of tyrosine kinase signaling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signaling pathways via. . .

SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis, stimulation of the immune system; treatment of retardation, acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed
wound
healing and psychosocial deprivation; treatment of pulmonary
dysfunction
and ventilator dependency; attenuation of **protein** **catabolic** responses after major surgery; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidio-blastosis; Adjuvant treatment for ovulation induction; stimulation of thymic development
and
prevention the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling and. . .

SUMM Phosphotyrosine recognition units/tyrosine phosphate recognition units/pTyr recognition units are defined as areas or domains of **proteins** or glycoproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not. . .

SUMM PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing **proteins** or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the. . .

SUMM . . . recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of

proteins or glycoproteins with pTyr recognition units (e.g. PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule with a **protein** or glyco-**protein** with pTyr recognition units either via a direct action on the pTyr recognition site or via an indirect mechanism. Examples. . . leading to initiation of normal or abnormal cellular activity; e) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to initiation of normal or abnormal cellular activity.

DETD . . . of dry nitrogen. The cooled reaction mixture was poured into a mixture of ice water (400 ml) and saturated aqueous **ammonium chloride** (100 ml) and extracted with ethyl acetate (3.times.200 ml). The combined organic extracts were washed with water (3.times.150 ml), dried. . .

DETD . . . diluted with water (100 ml), extracted with diethyl ether (2.times.100 ml). The combined organic extracts were washed with saturated aqueous **ammonium chloride** (2.times.100 ml), dried (MgSO.sub.4), filtered and evaporated in vacuo. The residue (1.8 g) was purified by column chromatography on silicagel. . .

DETD . . . ml) and extracted with ethyl acetate (2.times.50 ml). The combined organic extracts were washed with water (50 ml), saturated aqueous **ammonium chloride** (20 ml), dried (MgSO.sub.4), filtered and evaporated in vacuo. The residue was recrystallised from heptane affording after drying 1.55 g. . .

DETD . . . full-length sequence of PTP1B and the intracellular part of PTP.alpha. were introduced into the insect cell expression vector pVL1392. The **proteins** were expressed according to standard procedures. PTP1B was semi-purified by ion exchange chromatography, and PTP.alpha. was purified to apparent homogeneity. . .

L15 ANSWER 22 OF 109 USPATFULL

AB The present invention features calcilytic compounds. "Calcilytic compounds" refer to compounds able to inhibit calcium receptor activity.
Also described are the use of calcilytic compounds to inhibit calcium receptor activity and/or achieve a beneficial effect in a patient; and techniques which can be used to obtain additional calcilytic compounds.

AN 2000:15670 USPATFULL

TI Method of using calcilytic compounds

IN Del Mar, Eric G., Salt Lake City, UT, United States
Barmore, Robert M., Salt Lake City, UT, United States
Sheehan, Derek, Salt Lake City, UT, United States
Van Wagenen, Bradford C., Salt Lake City, UT, United States
Callahan, James F., Philadelphia, PA, United States
Keenan, Richard M., Malvern, PA, United States
Kotecha, Nikesh R., Thurmaston, United Kingdom
Lago, Maria Amparo, Audobon, PA, United States
Southall, Linda Sue, West Chester, PA, United States
Thompson, Mervyn, Harlow Essex, United Kingdom

PA NPS Pharmaceuticals, Inc., Salt Lake City, UT, United States (U.S. corporation)
SmithKline Beecham, Corp., Philadelphia, PA, United States (U.S. corporation)
SmithKline Beecham, PLC, Brentford, United Kingdom (non-U.S. corporation)

PI US 6022894 20000208 <--
AI US 1997-832984 19970404 (8)
RLI Continuation-in-part of Ser. No. US 1996-629608, filed on 9 Apr 1996,

now abandoned which is a continuation-in-part of Ser. No. US 1996-32263,
filed on 3 Dec 1996

PRAI US 1996-32263P 19961203 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Raymond, Richard L.
LREP Lyon & Lyon LLP
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3170
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6022894 20000208 <--

SUMM . . . calcium ions (Ca.sup.2+). Extracellular Ca.sup.2+ is under tight homeostatic control and regulates various processes such as blood clotting, nerve and **muscle** excitability, and proper bone formation.

SUMM Calcium receptor **proteins** enable certain specialized cells to respond to changes in extracellular Ca.sup.2+ concentration. For example, extracellular Ca.sup.2+ inhibits the secretion of. . . .

SUMM Extracellular Ca.sup.2+ acts directly on parathyroid cells to regulate PTH secretion. The existence of a parathyroid cell surface **protein** which detects changes in extracellular Ca.sup.2+ has been confirmed. (Brown et al., Nature 366:574, 1993.) In parathyroid cells, this **protein**, the calcium receptor, acts as a receptor for extracellular Ca.sup.2+, detects changes in the ion concentration of extracellular Ca.sup.2+, and. . . .

SUMM . . . of various molecules to mimic extracellular Ca.sup.2+ in vitro is discussed in references such as Nemeth et al., in "Calcium-Binding **Proteins** in Health and Disease," 1987, Academic Press, Inc., pp. 33-35; Brown et al., Endocrinology 128:3047, 1991; Chen et al., J.. . .

DETD (d) The transient increase is diminished by pretreatment with an activator of **protein** kinase C (PKC), such as phorbol myristate acetate (PMA), mezerein or (-)-indolactam V. The overall effect of the **protein** kinase C activator is to shift the concentration-response curve of calcium to the right without affecting the maximal response; and

DETD . . . duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, trophoblast in the placenta, platelet, vascular smooth **muscle** cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, endocrine and exocrine cells in the pancreas,. . . .

DETD . . . activity in different cells, such as a defective calcium receptor or an abnormal number of calcium receptors, a defective intracellular **protein** acted on by a calcium receptor, or a defective **protein** or an abnormal number of **proteins** acting on a calcium receptor.

DETD . . . extracellular Ca.sup.2+ as follows: elevated Ca.sup.2+ inhibits formation of 1,25(OH).sub.2 -vitamin D by proximal tubule cells, stimulates production of calcium-binding **protein** in distal tubule cells, and inhibits tubular reabsorption of Ca.sup.2+ and Mg.sup.2+ in the thick ascending limb of Henle's loop. . . .

DETD . . . occurs in cardiac arrest or neonatal distress, epilepsy, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and Parkinson's disease, dementia, **muscle** tension,

depression, anxiety, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, schizophrenia, neuroleptic malignant syndrome, and Tourette's syndrome; diseases involving excess. . .

DETD One way of treating osteoporosis is by altering PTH secretion. PTH can have a **catabolic** or an anabolic effect on bone. Whether PTH causes a **catabolic** effect or an anabolic effect seems to depend on how plasma levels of PTH are altered. When plasma levels of. . .

DETD . . . was added to the suspension and stirred for another 2 hours. The reaction was then partitioned between ether and saturated **ammonium chloride**. The ether layer was separated, washed with dilute HCl, water, and saturated brine, dried over anhydrous sodium sulfate, and concentrated.. . .

L15 ANSWER 23 OF 109 USPATFULL

AB In this disclosure, there are provided materials which completely degrade in the environment far more rapidly than pure synthetic plastics but which possesses the desirable properties of a thermoplastic: strength, impact resistance, stability to aqueous acid or base, and deformation at higher temperatures. There is provided a method for using the degradable plastic materials in preparing strong, moldable solids. There is further provided a method of making and applications for macromolecular, surface active agents that change the wetting behavior of lignin-containing materials. These surface active agents are used to provide a method of making and applications for synthetic polymers coupled to pieces of a vascular plant using macromolecular surface active agents.

AN 2000:4935 USPATFULL

TI Biodegradable plastics and composites from wood

IN Meister, John J., 31675 Westlady Rd., Beverly Hills, MI, United States 48025
Chen, Meng-Jiu, 901 St. Louis, Apt. #25, Ferndale, MI, United States 48220

PI US 6013774 20000111 <--

AI US 1998-162986 19980929 (9)

RLI Division of Ser. No. US 1997-942868, filed on 2 Oct 1997, now patented, Pat. No. US 5852069 which is a division of Ser. No. US 1995-400891, filed on 8 Mar 1995, now patented, Pat. No. US 5741875 which is a continuation-in-part of Ser. No. US 1993-80006, filed on 21 Jun 1993, now patented, Pat. No. US 5424382 which is a continuation-in-part of Ser. No. US 1991-789360, filed on 8 Nov 1991, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Truong, Duc

LREP Reising, Ethington, Barnes, Kisselle, Learman & McCulloch, P.C.

CLMN Number of Claims: 4

ECL Exemplary Claim: 1

DRWN 8 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 1976

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6013774 20000111 <--

DETD . . . cellulose, hemicellulose, and lignin, possibly contaminated with the inert "mineral" portion of the plant: starch, lipid, silica bodies, silica stegmata, **protein** bodies, and mucilage.

DETD TABLE 4

Some Halides Useful in Polymerization of Lignin Containing-Materials.

Calcium Chloride
Magnesium Chloride
Sodium Chloride
Potassium Chloride Lithium Chloride **Ammonium Chloride**
Calcium Bromide Magnesium Bromide Sodium Bromide
Potassium Bromide Lithium Bromide Ammonium Bromide
Calcium Fluoride Magnesium Fluoride Sodium Fluoride
Potassium Fluoride. . .

DETD . . . with brown rot fungus Gloeophyllum trabeum. Three of these fungi are white-rot species that attack and degrade woody materials by **catabolic** activity while the fourth fungus is a brown-rot that acts as a negative control since it attacks woody materials by. . .

L15 ANSWER 24 OF 109 USPATFULL

AB There are disclosed novel synthetic peptides of formula (I) ##STR1## where A, B, D, E, F, G, J, m, n, and p are defined in the specification. Compounds of formula (I) promote the release of growth hormone in humans and animals. Growth promoting compositions containing such compounds of formula (I) as the active ingredient, methods of stimulating the release of growth hormone, and the use of such compounds of formula (I) are also disclosed.

AN 2000:4820 USPATFULL
TI Compounds with growth hormone releasing properties
IN Lau, Jesper, Farum, Denmark
Peschke, Bernd, M.ang.l.o slashed.v, Denmark
Hansen, Thomas Kruse, Herlev, Denmark
Johansen, Nils Langeland, Copenhagen, Denmark
Ankersen, Michael, Frederiksberg, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 6013658 20000111 <--
AI US 1997-897239 19970717 (8)
RLI Continuation of Ser. No. WO 1996-DK45, filed on 26 Jan 1996
PRAI DK 1995-99 19950127
DK 1995-100 19950127
DK 1995-1083 19950928
DK 1995-1084 19950928
DK 1995-1372 19951204
DT Utility
FS Granted
EXNAM Primary Examiner: Chang, Ceila
LREP Zelson, Steve T., Rozek, Carol E
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3638
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6013658 20000111 <--

SUMM . . . of growing. In addition, growth hormone is known to have a number of effects on metabolic processes, e.g., stimulation of **protein** synthesis and free fatty acid mobilization and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism.. . .

SUMM In disorders or conditions where increased levels of growth hormone is desired, the **protein** nature of growth hormone makes anything but parenteral administration non-viable. Furthermore, other directly

acting natural secretagogues, e.g., GHRH and PACAP,. . .

SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids, prevention and treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating bone. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation, treatment of pulmonary dysfunction and ventilator dependency, attenuation of **protein catabolic** responses after major surgery, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis, adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function, treatment of immunosuppressed patients, improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal homeostasis in the frail elderly, stimulation of osteoblasts, bone remodelling and. . .

DETD . . . addition, the aluminium complex was added via cannula to the sulfone-anion solution. Cooling was maintained for 30 min. Then aqueous **ammonium chloride** (40 ml; 10%), water (200 ml) and dichloromethane (200 ml) were added. The phases were separated, the organic phase was. . .

DETD . . . ether, 22 mmol) was added dropwise. After addition, the solution was warmed to room temp. It was given onto 10% **ammonium chloride** solution in water (200 mL). The phases were separated. The aqueous phase was extracted with ethyl acetate (3.times.50 mL). The. . .

L15 ANSWER 25 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors and the method of preparing these compounds are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 1999:155927 USPATFULL

TI Steroid receptor modulator compounds and methods

IN Jones, Todd K., Solana Beach, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States
Edwards, James P., San Diego, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)

PI US 5994544 19991130 <--

AI US 1997-947413 19971008 (8)

RLI Division of Ser. No. US 1995-464360, filed on 5 Jun 1995, now patented, Pat. No. US 5693646 which is a continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Huang, Evelyn Mei

LREP Elmer, J. Scott, Respess, William L.

CLMN Number of Claims: 1

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 10956

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5994544 19991130 <--
SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds and IR and mimics the effect of the native. . .
DETD . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antanbolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.
DETD . . . The GR and MR active compounds and compositions of the present inception will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.
DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .
DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .
DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .
DETD . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .
DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . for the transcription-modulating activity of the target

IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g, [.sup.3. . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

DETD . . . concentration (nM), requivuired to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.

DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . .

L15 ANSWER 26 OF 109 USPATFULL

AB Methods and compositions, useful in the treatment of amyloidosis and conditions and diseases associated therewith, such as Alzheimer's Disease (AD). These methods involve administering to a subject a pharmaceutical composition including one or more agents which modulate APP catabolism and amyloid deposition. Accordingly, the methods and compositions are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs. The methods are based, at least in part, on modulating catabolism of APP in APP-containing cells through the use of a mobile ionophore, such as carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone, which does not substantially alter the viability of those cells.

AN 1999:141575 USPATFULL
TI Method and composition for modulating amyloidosis
IN Reiner, Peter B., Vancouver, Canada
Connop, Bruce P., Vancouver, Canada
PA The University of British Columbia, Vancouver, Canada (non-U.S. corporation)
PI US 5981168 19991109 <--
AI US 1998-80141 19980515 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Leary, Louise N.
LREP Lahive & Cockfield, LLP
CLMN Number of Claims: 69
ECL Exemplary Claim: 1
DRWN 17 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1184
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5981168 19991109 <--

SUMM . . . the walls of the cerebral microvasculature. The neurofibrillary tangles are composed of bundles of paired helical filaments containing hyperphosphorylated tau **protein** (Lee, V. M and Trojanowski, J. Q, The disordered Cytoskeleton in Alzheimer's disease, Curr. Opin. Neurobiol. 2:653 (1992)). The neuritic. . . of deposits of

proteinaceous material surrounding an amyloid core (Selkoe, D. J., "Normal and abnormal biology of the .beta.-amyloid precursor **protein**", Annu. Rev. Neurosci. 17:489-517 (1994)).

SUMM . . . of Alzheimer's disease has been shown to be caused by missense mutations in (at least) three genes: the amyloid precursor **protein** (APP) gene itself (Goate, A. et al., "Segregation of a missense mutation in the amyloid precursor **protein** gene with familial Alzheimer's disease", Nature 349:704-706 (1991) and Mullan, M. et al., "A pathogenic mutation for probable Alzheimer's disease. . . Alzheimer's disease type 3 gene", Nature 376:775-778 (1995)). The missense mutations in APP are located in the region of the **protein** where proteolytic cleavage normally occurs (see below), and expression of at least some of these mutants results in increased production of A.beta. (Citron, M. et al., "Mutation of the .beta.-amyloid precursor **protein** in familial Alzheimer's disease increases .beta.-amyloid production", Nature 360:672-674 (1992), Cai, X-D. et al., "Release of excess amyloid .beta. **protein** from a mutant amyloid .beta. **protein** precursor", Science 259:514-516 (1993) and Reaume, A. G. et al., "Enhanced amyloidogenic processing of the beta-amyloid precursor **protein** in gene-targeted mice bearing the Swedish familial Alzheimer's disease mutations and a humanized A.beta. sequence", J Biol. Chem. 271:23380-23388 (1996)).. . . has indicated that these mutations cause an increase in A.beta. secretion (Martins, R. N. et al., "High levels of amyloid-.beta. **protein** from S182 (Glu.sup.246) familial Alzheimer's cells", 7:217-220 (1995) and Scheuner, D. et al., "Secreted amyloid beta-**protein** similar to that in the senile plaques of Alzheimer's disease is increased in vivo by presenilin 1 and 2 and. . . exhibit neuritic plaques and age-dependent memory deficits (Games, D. et al., "Alzheimer-type neuropathology in transgenic mice overexpressing V717F .beta.-amyloid precursor **protein**", Nature 373:523-525 (1995); Masliah, E. et al., "Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F .beta.-amyloid precursor **protein** and Alzheimer's disease", J Neurosci. 16:5795-5811 (1996); Hsiao, K. et al., "Correlative memory deficits, A.beta. elevation, and amyloid plaques in. . . the extra copy of this chromosome accounts for the early appearance of amyloid plaques (Kang, J. et al., "The precursor **protein** of Alzheimer's disease amyloid A4 **protein** resembles a cell-surface receptor", Nature 325:733-736 (1987); Tanzi, R. E. et al., "Amyloid .beta. **protein** gene: cDNA, mRNA distribution and genetic linkage near the Alzheimer locus", Science 235:880-884 (1987)). Taken together with the evidence derived. . .

SUMM APP is expressed and constitutively catabolized in most cells. The dominant **catabolic** pathway appears to be cleavage of APP within the A.beta. sequence by an enzyme provisionally termed .alpha.-secretase, leading to release. . . respectively, followed by release of A.beta. into the extracellular space. Several different C-terminal fragments are produced as intermediates in APP **catabolic** processing; of particular interest is the production of an intracellular an 12 kDa C-terminal fragment (C100) which is produced following. . .

SUMM . . . disrupt intracellular pH and/or acidic organelles. For example, exposure of cells to the monovalent ionophore, monensin, or high concentrations of **ammonium chloride** (NH.sub.4 Cl)

been shown to decrease APP proteolytic processing accompanied by concomitant alterations in full-length cellular APP. Similarly, the vacuolar. . .

DETD . . . substantially altering the viability of those cells. Modulation of, e.g., reducing, APP catabolism, results in a decreased production of amyloid-.beta. **protein** (A.beta.) or increasing production of soluble amyloid precursor **protein** (APP.sub.S), which are associated with amyloidosis and conditions related thereto, such as Alzheimer's disease.

DETD . . . the present invention can modulate amyloidosis in a subject such as by modulating APP catabolism, thereby decreasing production of amyloid-.beta. **protein** (A.beta.) or increasing production of soluble amyloid precursor **protein** (APP.sub.S).

DETD . . . is prevented or decreased. This modulation can be by one or more chemically induced physiological mechanisms. For example, the dominant **catabolic** pathway appears to be cleavage of APP within the A.beta. sequence by an enzyme provisionally termed .alpha.-secretase, leading to release. . . respectively, followed by release of A.beta. into the extracellular space. Several different C-terminal fragments are produced as intermediates in APP **catabolic** processing; such as the production of an intracellular an 12 kDa C-terminal fragment (C100) which is produced following .beta.-secretase activity. . .

DETD Detection of Intra- and Extracellular APP **Catabolic** Fragments

DETD . . . fragments, the media was retained and centrifulged at 4.degree. C. for 10 min at 16,000.times.g to remove cellular debris. Extracellular **protein** was then precipitated by 10% trichloroacetic acid as previously described (31). Subsequently, secreted APPs.alpha. was determined by 10% Tris-Glycine SDS-PAGE. . . of intracellular APP fragments, cultures were harvested in ice-cold lysis buffer and then sonicated for 8 seconds on ice. Cellular **protein** levels were quantified using the BCA **protein** assay (Pierce, Rockford, Ill.) and 10 .mu.g of cellular **protein** were then separated by either 10% Tris-Glycine or 16% Tris-Tricine SDS-PAGE. Intracellular C100 and total APP were quantitated, respectively, by. . . and 10% Tris-Glycine Western Blot analysis using an anti-APP N-terminal antibody (22C11, Boehringer Mannheim, Laval, QC). A diagram of the **catabolic** fragments of APP and the epitopes to which each antibody binds is depicted in FIG. 1. As seen in FIG.. . .

DETD Cellular (C100 and total APP) and extracellular (APPs.alpha. and A.beta.) APP **catabolic** fragments were separated using SDS PAGE with either 10% Tris-Glycine (for APPs.alpha. and total APP) or 16% Tris-Tricine (for C100 and A.beta.) gels. Following electrophoretic separation, **proteins** were transferred to nitrocellulose membranes and probed with either the monoclonal antibody WO-2 to detect C100, APPs.alpha. and A.beta., or. . .

DETD . . . an Optocomp.RTM. II luminometer (MGM Instruments, Hamden, Conn.) and ATP levels were determined by extrapolation from a standard curve. Cellular **protein** levels were quantified using the BCA **protein** assay (Pierce, Rockford, Ill.) and cellular ATP levels were expressed as .mu.mol/.mu.g **protein**. As a positive control, the effect of combined treatment with various concentrations of sodium azide and 2-deoxy-D-glucose (NaA/DG) was also. . .

DETD . . . illustrates the effect of FCCP exposure to K695 cells at various concentrations for 30 minutes and cellular and released APP **catabolic** fragments quantified by Western blot analysis. The effect of FCCP on A) A.beta. release, B) cellular C100, C) APPs.alpha. release. . .

DETD . . . 3D). FIG. 3 illlustrates the effect of FCCP exposure at various concentrations for 4 hours and cellular and released APP **catabolic** fragments quantified by Western blot analysis. The effect of FCCP on A) A.beta. release, B) cellular C100, C) APPs.alpha. release. . .

DETD . . . APPs.alpha. production. The effect of FCCP upon A.beta. and APPs.alpha. production did not appear to be secondary to effects upon **protein** maturation, as the both total levels of cellular APP as well as maturation of APP were unaffected (FIG. 4C). Because. . . low levels of C100 found in K695 cells, we were unable to quantify the effects of FCCP upon this APP **catabolic** fragment. FIG. 4 illustrates the effect of FCCP exposure at various concentrations for 4 hours and cellular and released APP **catabolic** fragments quantified by Western blot analysis. The effect of FCCP on A) A.beta. release, B) cellular C100, and C) total. . .

DETD The abbreviations used hereinabove are: AD, Alzheimer's Disease; A.beta., amyloid .beta. peptide; APP, amyloid precursor **protein** ; APPs.alpha., .alpha.-secretase cleaved N-terminal ectodomain of APP; baf A1, bafilomycin A1; C100, C-terminal fragment containing the amyloid-.beta. sequence; DG, 2-deoxy-D-glucose;. . .

CLM What is claimed is:

1. A method of decreasing amyloid precursor **protein** (APP) catabolism, comprising the step of administering to APP-containing cells an amount of a mobile ionophore composition effective to prevent. . .

13. A method of treating Alzheimer's disease, comprising the step of administering to a subject having amyloid precursor **protein** (APP)-containing cells a pharmaceutically effective amount of a mobile ionophore composition effective to prevent or decrease APP catabolism without substantially. . .

. . . . method of treating a disease state associated with amyloidosis, comprising the step of administering to a subject having amyloid precursor **protein** (APP)-containing cells a pharmaceutically effective amount of a mobile ionophore composition effective to prevent or decrease APP catabolism without substantially. . .

. . . . associated with amyloidosis, comprising a therapeutically effective amount of a mobile ionophore composition effective to prevent or decrease amyloid precursor **protein** (APP) catabolism in APP-containing cells without substantially altering the viability of said APP-containing cells, and a pharmaceutically acceptable vehicle.

53. A method for decreasing amyloid deposition in a subject, comprising administering to a subject having amyloid precursor **protein** (APP)-containing cells an effective amount of a mobile ionophore composition effective to prevent or decrease APP catabolism without substantially altering. . .

65. A method of decreasing amyloid precursor **protein** (APP) proteolysis or secretion of fragments thereof, comprising the step of administering to APP-containing cells an amount of a mobile. . .

66. A method of treating Alzheimer's disease, comprising the step of administering to a subject having amyloid precursor **protein** (APP)-containing cells a pharmaceutically effective amount of a mobile

ionophore composition effective to prevent or decrease APP proteolysis or secretion. . .
. . . method of treating a disease state associated with amyloidosis, comprising the step of administering to a subject having amyloid precursor **protein** (APP)-containing cells a pharmaceutically effective amount of a mobile ionophore composition effective to prevent or decrease APP proteolysis or secretion. . .
. . . associated with amyloidosis, comprising a therapeutically effective amount of a mobile ionophore composition effective to prevent or decrease amyloid precursor **protein** (APP) proteolysis in or to prevent or decrease APP fragment secretion from APP-containing cells without substantially altering the viability of. . .
69. A method for decreasing amyloid deposition in a subject, comprising administering to a subject having amyloid precursor **protein** (APP)-containing cells an effective amount of a mobile ionophore composition effective to prevent or decrease APP proteolysis or APP fragment. . .

L15 ANSWER 27 OF 109 USPATFULL
AB Compounds of peptide mimetic nature having the general formula I ##STR1## wherein a and b are independently 1 or 2, R.sup.1 and R.sup.2 are independently H or C.sub.1-6 alkyl, G and J are independently, inter alia, aromats, and D and E are independently several different groups are growth hormone secretagogous with improved bioavailability.
AN 1999:137327 USPATFULL
TI Compounds with growth hormone releasing properties
IN Hansen, Thomas Kruse, Herlev, Denmark
Peschke, Bernd, Maaloev, Denmark
Lau, Jesper, Farum, Denmark
Lundt, Behrend Friedrich, Kokkedal, Denmark
Ankersen, Michael, Frederiksberg, Denmark
Watson, Brett, Vaerloese, Denmark
Madsen, Kjeld, Vaerloese, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 5977178 19991102 <--
AI US 1996-769020 19961218 (8)
PRAI US 1996-22062P 19960722 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Rotman, Alan L.; Assistant Examiner: Aulakh, Charanjit S.
LREP Zelson, Steve T., Lambiris, Elias J., Rozek, Carol E.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 7142
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5977178 19991102 <--
SUMM . . . of growing. In addition, growth hormone is known to have a number of effects on metabolic processes, e.g., stimulation of **protein** synthesis and free fatty acid mobilisation and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism.. . .
SUMM In disorders or conditions where increased levels of growth hormone is desired, the **protein** nature of growth hormone makes anything but parenteral administration non-viable. Furthermore, other directly acting natural secretagogues, e.g., GHRH and PACAP,. . .
SUMM . . . The uses of growth hormone may be summarized as follows:

stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids, prevention and treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating borte. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation, treatment of pulmonary dysfunction and ventilator dependency, attenuation of **protein catabolic** responses after major surgery, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis, adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function, treatment of immunosuppressed patients, improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal homeostasis in the frail elderly, stimulation of osteoblasts, bone remodelling and. . .

DETD . . . mixture was stirred for 1.5 h at -78.degree. C. and then warmed to room temperature. A 10% aqueous solution of **ammonium chloride** (200 ml) was added dropwise. The phases were separated. The aqueous phase was extracted with ethyl acetate (3.times.100 ml). The. . .

DETD . . . completed, the solution was heated to reflux for 16 h. It was cooled to 5.degree. C. A 10% solution of **ammonium chloride** in water (60 ml) was added dropwise. The solution was warmed to 50.degree. C. for 1 h. It was cooled. . .

L15 ANSWER 28 OF 109 USPATFULL

AB The present invention relates to novel organic compounds, to methods for their preparation, to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are compounds of formula (I)

(L).sub.n --Ar.sub.1 --R.sub.1 A (I)

wherein

(L).sub.n, n, Ar.sub.1, R.sub.1 and A are as defined in the application.

AN 1999:132860 USPATFULL
TI Modulators of molecules with phosphotyrosine recognition units
IN Andersen, Henrik Sune, Kobenhavn, Denmark
Moller, Niels Peter Hundahl, Kobenhavn, Denmark
Madsen, Peter, Bagsvaerd, Denmark
PA Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S. corporation)
PI US 5972978 19991026 <--
AI US 1999-252883 19990219 (9)
RLI Division of Ser. No. US 1997-842801, filed on 16 Apr 1997
PRAI DK 1996-464 19960419
US 1996-22116P 19960717 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Richter, Johann; Assistant Examiner: Oswecki, Jane C.

LREP   Zelson, Steve T., Rozek, Carol E.
CLMN   Number of Claims: 9
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 2078
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5972978   19991026   <--

AB . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are. . .

SUMM . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings.

SUMM Phosphorylation of **proteins** is a fundamental mechanism for regulation of many cellular processes. Although **protein** phosphorylation at serine and threonine residues quantitatively dominating in eukaryotic cells, reversible tyrosine phosphorylation seems play a pivotal role in. . .

SUMM The regulation of **protein** tyrosine phosphorylabon in vivo is mediated by the opposing actions of **protein** tyrosine kinases (PTKs) and **protein** tyrosine phosphatases (PTPases). The level of **protein** tyrosine phosphorylation of cellular **proteins** is determined by the balanced activities of PTKs and PTPase (Hunter, 1995, supra).

SUMM The **protein** phosphatases are composed of at least two separate and distinct families (Hunter, T., Cell 58: 1013-1016 (1989)) the **protein** serine/threonine phosphatases and the PTPases.

SUMM Low molecular weight phosphotyrosine-**protein** phosphatase (LMW-PTPase) shows very little sequence identity to the intracellular PTPases described above. However, this enzyme belongs to the PTPase. . .

SUMM . . . more than 500 different species will be found in the human genome, i.e. close to the predicted size of the **protein** tyrosine kinase superfamily (Hanks and Hunter, FASEB J. 9: 576-596 (1995)).

SUMM PTPases are the biological counterparts to **protein** tyrosine kinases (PTKs). Therefore, one important function of PTPases is to control, down-regulate, the activity of PTKs. However, a more. . .

SUMM Dual specificity **protein** tyrosine phosphatases (dsPTPases) define a subclass within the PTPases family that can hydrolyze phosphate from phosphortyrosine as well as from. . . PTPases: His-Cys-Xx-Xx-Gly-Xxx-Xxx-Arg. (SEQ ID NO:2) At least three dsPTPases have been shown to dephosphorylate and inactivate extracellular signal-regulated kinase (ERKs)/mitogen-activated **protein** kinase (MAPK): MAPK phosphatase (CL100, 3CH134) (Charles et al., Proc. Natl. Acad. Sci. USA 90: 5292-5296 (1993)); PAC-1 (Ward et. . .

SUMM . . . domains and PTB domains primarily act as docking molecules with little or no catalytic activity. In other words, tyrosine phosphorylated

proteins have the capacity to bind other **proteins** containing SH2 domains or PTB domains thereby controlling the subcellular location of signalling molecules. There appears to be a significant. . .

SUMM In an early study, vanadate was found to inhibit **protein** -tyrosine phosphatases in mammalian cells with a concomitant increase in the level of phosphotyrosine in cellular **proteins** leading to transformation (Klarlund, Cell 41: 707-717 (1985)). Vanadium-based phosphatase inhibitors are relatively unspecific. Therefore, to assess the importance of. . .

SUMM . . . Mooney and Anderson, J. Biol. Chem. 264: 6850-6857 (1989)), with the tri-phosphorylated tyrosine-1150 domain being the most sensitive target for **protein**-tyrosine phosphatases (PTPases) as compared to the di- and mono- phosphorylated forms (King et al., Biochem. J. 275: 413-418 (1991)). It. . .

SUMM . . . be obtained in adipocytes (Fantus et al., Biochemistry 28: 8864-8871 (1989); Eriksson et al., Diabetologia 39: 235-242 (1995)) and skeletal **muscle** (Leighton et al., Biochem. J. 276: 289-292 (1991)). In addition, recent studies show that a new class of peroxovanadium compounds. . .

SUMM . . . signalling in a rat hepatoma cell line (Kulas et al., J. Biol. Chem. 270: 2435-2438 (1995)). A suppression of LAR **protein** levels by about 60 percent was paralleled by an approximately 150 percent increase in insulin-induced autophosphorylabon. However, only a modest. . .

SUMM . . . the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family **protein** -tyrosine kinase (Mustelin et al., Proc. Natl. Acad. Sci. USA 86: 6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. USA. . . to T-cell activation. In a recent study it was found that recombinant p56.sup.lck specifically associates with recombinant CD45 cytoplasmic domain **protein**, but not to the cytoplasmic domain of the related PTP.alpha. (Ng et al., J. Biol. Chem. 271: 1295-1300 (1996)). The. . . mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosine. In immature B cells, another member of the Src family **protein**-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri et al., J. Biol.. . .

SUMM . . . fibroblasts grow on appropriate substrates, seem to mimic, at least in part, cells and their natural surroundings. Several focal adhesion **proteins** are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, Neuron 11, 551-564 (1993)). However, aberrant tyrosine phosphorylaton of these **proteins** can lead to cellular transformation. The intimate association between PTPases and focal adhesions is supported by the finding of several. . . PTPD1 (M.o slashed.ller et al., Proc. Natl. Acad. Sci. USA 91: 7477-7481 (1994)). The ezrin-like domain show similarity to several **proteins** that are believed to act as links between the cell membrane and the cytoskeleton. PTPD1 was found to be phosphorylated. . .

SUMM PTPases may oppose the action of tyrosine kinases, inducing those responsible for phosphorylation of focal adhesion **proteins**, and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool et. . .

SUMM . . . al., J. Biol. Chem. 260: 4653-4660 (1985); Lau et al., J. Biol.

Chem. 262: 1389-1397 (1987); Lau et al., Adv. **Protein** Phosphatases 4: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when. . .

SUMM In preferred embodiments, the compounds of the invention modulate the activity of **protein** tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s).

SUMM In one preferred embodiment the compounds of the invention act as inhibitors of PTPases, e.g. **protein** tyrosine phosphatases involved in regulation of tyrosine kinase signalling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signalling pathways via. . .

SUMM . . . The uses of growth hormone may be summarized as follows: stmulabon of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis, stimulation of the immune system; treatment of retardation, acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depressions, Alzheimers disease, delayed wound healing and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein** **catabolic** responses after major surgery; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidio-blastosis; Adjuvant treatment for ovulation induction; simulation of thymic development and prevention the age-related decline of thymic function; treatment of immunosuppressed patents; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling and. . .

SUMM Phosphotyrosine recognition units/tyrosine phosphate recognition units/pTyr recognition units are defined as areas or domains of **proteins** or glycoproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not. . .

SUMM PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing **proteins** or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the. . .

SUMM . . . recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of **proteins** or glycoproteins with pTyr recognition units (e.g. PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule with a **protein** or glyco-**protein** with pTyr recognition units either via a direct action on the ptyr recognition site or via an indirect mechanism. Examples. . . leading to initiation of normal or abnormal cellular activity; e) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with ptyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with ptyr leading to initiation of normal or abnormal cellular activity.

DETD A mixture of the above acetonitrile (2.50 g, 15 mmol), **ammonium chloride** (1.60 g, 30 mmol) and sodium azide (1.94 g, 30 mmol) in N,N'-dimethylformamide (25 ml) was stirred at 125.degree. C.. . .

DETD A mixture of the above acetonitrile (5.40 g, 32 mmol), **ammonium chloride** (2.59 g, 48 mmol) and sodium azide (3.15 g, 48 mmol) in N,N'-dimethylformamide (100 ml) was stirred at 125.degree. C.. . .

DETD . . . full-length sequence of PTP1B and the intracellular part of

PTP.alpha. were introduced into the insect cell expression vector pVL1392. The **proteins** were expressed according to standard procedures. PTP1B was semi-purified by ion exchange chromatography, and PTP.alpha. was purified to apparent homogeneity. . . .

L15 ANSWER 29 OF 109 USPATFULL

AB The ATP-ubiquitin-dependent process has been shown to be responsible for the excessive **protein** degradation which occurs in conditions or disease states in which there is severe loss of body mass and negative nitrogen balance has been identified and key constituents in the process identified. A method of inhibiting the accelerated or enhanced proteolysis, a method of identifying inhibitors of the process, multipain and the proteasome inhibitor are the subject of the claimed invention.

AN 1999:132526 USPATFULL

TI ATP-dependent protease and use of inhibitors for same in the treatment of cachexia and **muscle** wasting

IN Goldberg, Alfred L., Brookline, MA, United States

PA The President and Fellows of Harvard College, Cambridge, MA, United States (U.S. corporation)

PI US 5972636 19991026 <--

AI US 1997-982295 19971202 (8)

RLI Division of Ser. No. US 1996-730310, filed on 11 Oct 1996, now patented, Pat. No. US 5786329 which is a division of Ser. No. US 1994-262497, filed on 20 Jun 1994, now patented, Pat. No. US 5565351 which is a division of Ser. No. US 1991-699184, filed on 13 May 1991, now patented, Pat. No. US 5340736

DT Utility

FS Granted

EXNAM Primary Examiner: Patterson, Jr., Charles L.

LREP Sterne, Kessler, Goldstein & Fox P.L.L.C.

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 12 Drawing Page(s)

LN.CNT 2944

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI ATP-dependent protease and use of inhibitors for same in the treatment of cachexia and **muscle** wasting

PI US 5972636 19991026 <--

AB The ATP-ubiquitin-dependent process has been shown to be responsible for the excessive **protein** degradation which occurs in conditions or disease states in which there is severe loss of body mass and negative nitrogen. . . .

SUMM Mammalian cells contain at least four proteolytic systems which appear to serve distinct functions in the turnover of cell **proteins**. In the cytosol, there is a soluble proteolytic pathway that requires ATP and involves the polypeptide ubiquitin. This multicomponent system catalyzes the selective degradation of highly abnormal **proteins** and short-lived regulatory **proteins**. However, this process also appears to be responsible for the breakdown of most **proteins** in maturing reticulocytes. Boches, F. and A. L. Goldberg, Science, 215:978-980 (1982); Spenser, S. and J. Etlinger, J. Biol. Chem.,. . . et al., J. Biol. Chem., 260:3344-3349 (1985) In cells deprived of insulin or serum, the breakdown of the average cell

proteins increases up to 2-fold. This accelerated proteolysis involves the lysosomes, which are also the sites for the breakdown of endocytosed and membrane **protein**. Another system by which skeletal **muscle** can increase overall proteolysis involves the Ca.sup.2+ -dependent proteases (calpains I and II). In dystrophic or damaged **muscle** or in normal **muscle** after treatments that raise intracellular Ca.sup.+, overall **protein** breakdown rises, due mainly to activation of the calpains. In addition, there is a nonlysosomal degradative system that functions independently of ATP; in erythrocytes, this system catalyzes the selective breakdown of oxidant-damaged **proteins**. The relative importance of these systems in the degradation of different cell components under various conditions in **muscle** is unknown.

SUMM In the process requiring Ub, the first step in degradation of many **proteins** involves their conjugation to this small polypeptide by an ATP-requiring process. The ubiquitinated **proteins** are then degraded by a 1000-1500 kDa (26S) ATP-dependent proteolytic complex, the Ub-Conjugate-Degrading Enzyme ("UCDEN"). This pathway has been best characterized in reticulocytes, but has also been demonstrated in skeletal **muscle** and other cells. It is believed to be responsible for the rapid degradation of highly abnormal **proteins** and many short-lived enzymes or regulatory **proteins**.

SUMM . . . contains 12-15 distinct subunits and three distinct peptidases of different specificities. By itself, the proteasome is unable to degrade ubiquitinated **proteins** and provides most of the proteolytic activity of UCDEN.

SUMM The present invention-relates to a method of inhibiting (reducing or preventing) the accelerated breakdown of **muscle proteins** which accompanies various physiological and pathological states and is responsible to a large extent for the loss of **muscle** mass (atrophy) which follows nerve injury, fasting, fever, acidosis and certain endocrinopathies. As described herein, it has been shown that the nonlysosomal ATP-ubiquitin-dependent proteolytic process increases in **muscle** in these conditions and is responsible for most of the accelerated proteolysis which occurs in atrophying **muscles**. This is supported by the demonstration, also described herein, that there is a specific increase in ubiquitin mRNA, induction of mRNA for proteasome and increased ubiquitinated **protein** content in atrophying **muscles** which is not seen in non-**muscle** tissue under the same conditions.

SUMM The present invention further relates to a novel ATP-dependent protease which is involved in degradation of ubiquitinated **proteins**, forms a complex with the proteasome and appears to be part of the 1300-1500 kDa ATP-dependent proteolytic complex (UCDEN referred to as the 1500 kDa complex) which rapidly degrades **proteins** conjugated to ubiquitin. This novel protease, referred to as multipain, appears to play a critical role in the ATP-ubiquitin-dependent pathway.

SUMM Multipain is a multimeric enzyme of molecular weight approximately 500 kDa, which requires ATP hydrolysis for activation and degrades ubiquitinated **proteins** preferentially. This new ATP-dependent enzyme appears to be a thiol protease and has been shown to cleave Ub-conjugated **proteins** to acid-soluble products. Multipain has been identified in **muscle** and shown to play an essential role in the cytosolic pathway which is activated in various forms of **muscle** wasting.

SUMM The present invention further relates to purified multipain, obtained from sources in which it normally is found, such as skeletal **muscle** cells; DNA or RNA encoding multipain; multipain produced by recombinant DNA methods; antibodies specific for the enzyme; methods of using multipain; and multipain inhibitors and their use, particularly for reducing the loss of **muscle** mass which occurs in a variety of diseases or conditions.

SUMM . . . an inhibitor of another component of the 1500 kDa complex can be administered to an individual in whom loss of **muscle** mass occurs (e.g., following nerve injury, fasting, infection or certain endocrinopathies). **Muscle** mass losses in such conditions are due in turn to accelerated breakdown of **muscle proteins**, which has been shown, as described herein, to be due largely to activation of the non-lysosomal ATP-ubiquitin-dependent pathway, in which. . . a multipain inhibitor or an inhibitor of another component of the ATP-dependent proteolytic complex will interfere with or reduce enhanced **protein** breakdown which normally occurs in such conditions. As a result, proteolysis is reduced and **muscle protein** loss occurs to a lesser extent than normally occurs in such conditions. This method of inhibiting multipain or another component of the 1500 kDa complex and, as a result, of inhibiting destruction of **muscle protein**, can be used in a wide variety of conditions, such as cancer, chronic infectious diseases, fever and **muscle** disuse and denervation, in which it occurs and often can be extremely debilitating. The method is also useful in conditions. . .

DRWD FIG. 1 is a graphic representation of the results of fractionation of extracts from rabbit skeletal **muscle** fraction II by mono-Q anion exchange chromatography. Subsequent analysis focused on peak 2 because it was shown, as described herein,. . .

DRWD . . . protease, in which the peak of activity degrading Ub-.sup.125 I-lysozyme from the Superose 6 column was concentrated, and 25 .mu.g **protein** was analyzed.

DRWD FIG. 9 is a graphic representation of the effect of ATP-depletion on **protein** breakdown in denervated and normal soleus **muscles**. These data show that overall proteolysis increases primarily by activation of the ATP-dependent pathway following denervation. Values are the means.+-.the. . . for at least 5 rats in which both sciatic nerves were cut, or for unoperated normal rats. Upper Left: Total **protein** degradation on each day after cutting the sciatic nerve and in normal **muscles** from rats of similar size (60-70 g), Upper Right: Effect of ATF-depletion on rates of proteolysis. Lower Left: The relative changes in total **protein** breakdown and in the energy-independent proteolytic process after denervation (i.e., the difference in means rates of proteolysis between denervated **muscles** and normal ones). Lower Right: The relative changes in the ATP-dependent process after denervation.

DRWD FIG. 10 is a graphic representation of the effects of fasting and refeeding on **protein** breakdown in rat extensor digitorum longus **muscle**. Left panel: Total **protein** breakdown and the energy independent process in **muscles** from fed or fasted rats were measured at different times after removal of food and 24 hours after refeeding. Right panel: The ATP-independent component of **protein** breakdown. Values are the means.+-.the SEM for 6 rats.

DRWD FIG. 11 shows results of Northern blot analysis Ub mRNA in

muscle from fasting and fasted-refed rats. Shown are levels of polyUb mRNA in 10 .mu.g of total RNA/lane isolated from soleus **muscle** of fed rats (a) and fasted rats for 24 hrs. (b) 48 hrs. (c) or fasted 48 hrs. and refed. . . .

DRWD FIG. 12 is a graphic representation of levels of total mRNA determined by dot blot analysis in soleus **muscles** of fasted and fasted-refed rats, as described in Example 6. Significant difference from fed animals, *p<0.005, **p<0.05.

DETD The present invention is based on the identification of the pathway responsible for the excessive **protein** degradation which occurs in conditions or disease states in which there is severe loss of body mass (e.g., cachexia) and. . . of constituents of this pathway, which make it possible to inhibit the pathway and the negative nitrogen balance in these **catabolic** states.

DETD As described herein, work undertaken to learn which of the proteolytic systems is responsible for the large increase in **protein** breakdown in skeletal **muscle** during denervation atrophy, fasting and other **catabolic** states (e.g., fever) has shown that most of the accelerated proteolysis in **muscle** in fasting or denervation atrophy is due to activation of the nonlysosomal (cytosolic) ATP-ubiquitin-dependent proteolyte process, which until now has been generally believed to be a constitutive process (often termed "basal **protein** breakdown") and to be primarily responsible for the elimination of abnormal or short-lived regulatory polypeptides. As described herein, however, it has been shown that there is a specific cellular response which leads to loss of **muscle protein** and is triggered by a variety of physiological and pathological stimuli. For example, in fasting, the enhancement of **muscle protein** breakdown requires glucocorticoids and low insulin and in febrile infections, requires interleukin-1 and TNF. As is also described herein, ubiquitin is critical in enhancing the activity of the nonlysosomal ATP-dependent process in **muscle** in denervation atrophy, fasting, and treatment with hormones or endotoxin.

DETD It is possible that multiple steps in the ATP-Ub-dependent pathway are affected in **muscle** by fasting and denervation, but the work described herein has resulted in isolation of a new, rate-limiting component in the large (1500 kDa) enzyme complex which hydrolyzes cell **protein** which are marked for degradation by covalent linkage to the cofactor ubiquitin. Thus, the work described herein has identified a key target for inhibition. As described, a protease has been identified in **muscle** and has been shown to play an essential role in the cytosolic ATP-ubiquitin-dependent proteolytic pathway now known to be activated in various forms of **muscle** wasting. As further described, a polypeptide inhibitor of the proteasome's degradative activities has also been identified.

DETD . . . present invention relates to a method of inhibiting (reducing or preventing) the accelerated or enhanced proteolysis which occurs in atrophying **muscles** and is now known to be due to activation of the nonlysosomal ATP-requiring process in which ubiquitin plays a critical. . . is inhibited by interfering with the ATP-Ub-dependent pathway at one or more possible steps (e.g., by reducing ubiquitin conjugation of **proteins**, by interfering with activity of UCDEN, or by interfering with activity of one of its components, such as the novel. . .

DETD The present invention also relates to the discovery in **muscle** of the protease which requires ATP hydrolysis for function and has an

essential role in the cytosolic ATP-ubiquitin-dependent proteolytic pathway activated in various forms of **muscle** wasting. This proteolytic enzyme, called "multipain", is a 500 kDa multimer or **protein** complex which appears to be a thiol protease related to the papain family of proteases. It contains 6 or nimre high molecular weight subunits (50-130 kDa in size) and has been shown to degrade ubiquitin-conjugated **proteins** preferentially, by an ATP-dependent reaction. A variety of observations, also described herein, indicate that this protease is the rate limiting component in the recognition and degradation of **proteins** conjugated to ubiquitin. Multipain also has the ability to depolymerize the multiple-ubiquitin chain by an isopeptidase activity. It is sensitive. . .

DETD Thus, inhibition of the ATP-ubiquitin-dependent pathway is a new approach for treating the negative nitrogen balance in **catabolic** states. This can be effected, for example, through use of an inhibitor of the newly discovered proteolytic enzyme, resulting in reduction of loss of **muscle** mass in conditions in which it occurs. Such an inhibitor can also be used in reducing the activity of the cytosolic ATP-ubiquitin-dependent proteolytic system in cell types other than **muscle** cells. Excessive **protein** loss is common in many types of patients, including individuals with sepsis, burns, trauma, many cancers, chronic or systemic infections,. . . in individuals receiving corticosteriods, and those in whom food intake is reduced and/or absorption is compromised. Moreover, inhibitors of the **protein** breakdown pathway could possibly be valuable in animals (e.g., for combating "shipping fever", which often leads to a major weight. . .

DETD The following is a description of the work which led to the discovery that most of the accelerated proteolysis in **muscle** in these conditions is due to activation of the nonlysosomal ATP-requiring process; isolation and characterization of the protease multipain; its. . . of identifying multipain inhibitors and inhibitors identified by these methods and a method of inhibiting multipain and its effect on **muscle** degradation.

DETD Demonstration That the Cytosolic ATP-Dependent Proteolytic Pathway Is Critical in Atrophy of Skeletal **Muscle**

DETD As described herein, particularly in Examples 3-5, assessment of whether the accelerated proteolysis evident in atrophy of skeletal **muscles** upon denervation or fasting is catalyzed by the nonlysosomal ATP-dependent or energy-independent degradative systems has been carried out. This work has clearly demonstrated a link between the nonlysosomal ATP-dependent pathway and **muscle** wasting. As described herein, it has been shown that in a variety of **catabolic** states (e.g., denervation, fasting, fever, certain endocrinopathies or metabolic acidosis) **muscle** wasting is due primarily to accelerated **protein** breakdown and, in addition, that the increased proteolysis results from activation of the cytosolic ATP-ubiquitin-dependent proteolytic system, which previously had been believed to serve only in the rapid elimination of abnormal **proteins** and certain short-lived enzymes. The discovery that this pathway is responsible for the accelerated proteolysis in these **catabolic** states is based on studies in which different proteolytic pathways were blocked or measured selectively in incubated **muscles**, and the finding of increased mRNA for components of this pathway (e.g. for ubiquitin and proteasome subunits) and increased levels of ubiquitin-**protein** conjugates in the atrophying **muscles**. As described herein, simple animal models that closely

mimic these **catabolic** states (e.g., disuse, atrophy, sepsis, endotoxin-treatment, which mimics fever and muscular dystrophy) have been developed, as have methods for precise measurement of rates of **protein** breakdown in **muscles** during in vitro incubations.

DETD Results showed that when normal skeletal **muscles** incubated in vitro were depleted almost completely of ATP, **protein** breakdown decreased by 40-70%. The ATP-dependent (nonlysosomal) proteolytic process was found to be measured specifically and reproducibly if the residual ATP-dependent process was subtracted from the total **protein** breakdown seen in the control contralateral **muscle**. Within 1 and 3 days after denervation of the soleus, this ATP-dependent process increased by 50-250%, while the residual (energy-independent) process did not change. The rise in this ATP-dependent, nonlysosomal process accounted for all of the increased **protein** breakdown during denervation atrophy, including the rapid degradation of actin (as shown by increased 3-methylhistidine production). This response again accounted for most of the enhanced **protein** breakdown in fasting.

DETD After food deprivation, ATP-dependent proteolysis in the **muscles** increased selectively by 150-350%. After refeeding, this process returned to control levels within 1 day. In addition, in **muscle** extracts from fasted rabbits, the ATP-dependent degradation of endogenous **proteins** and .sup.14 C-casein was about 2-fold faster than in extracts from fed animals. Similarly, selective increase in ATP-dependent proteolysis in **muscles** occurred in sepsis, as modeled by the injection of endotoxin (LPS).

DETD Thus, as shown herein, the increase in the ATP-dependent process in **muscle** is a specific cellular response, activated in a variety of **catabolic** states, which appears responsible for most of the accelerated proteolysis in atrophying **muscles**. The conditions which influence the ATP-requiring degradative system include-denervation atrophy, fasting, fever, certain endocrinopathies and acidosis.

DETD Activation of the ATP-Ubiquitin-Dependent System in **Muscle** During Fasting and Denervation Atrophy

DETD As described above, activation of an ATP-dependent proteolytic process appears responsible for most of the increased **protein** degradation in skeletal **muscle** during fasting and denervation atrophy. Because this process might involve the activation of the ATP-ubiquitin-dependent pathway, the levels of mRNA for ubiquitin (Ub) and Ub **protein** content in such atrophying **muscles** were measured (See Example 6). After food deprivation of rats for 1 day, a 2- to 4-fold increase in the levels of two polyUbiquitin transcripts (2.4 and 1.3 kDa) was detected in the soleus and extensor digitorium longus **muscles**, although their total RNA and total mRNA content fell by 50%. After denervation of the soleus, a 2- to 3-fold.

.

. Ub mRNA upon fasting or denervation was accompanied by a 60-90% rise in the total content of ubiquitin in these **muscles**. When fasted animals were refed, the levels of Ub mRNA in their **muscles** returned to control levels within 1 day.

DETD As discussed above, degradation of many **proteins** in eukaryotic cells involves their conjugation to a small polypeptide, ubiquitin, by an ATP-requiring process. UCDEN (Ub-Conjugate Degrading Enzyme or megapain) degrades the ubiquitinated **proteins**. The precise nature of UCDEN is unclear, although it has been shown that the 1000-1500 kDa (26S) complex can be. . .

DETD As described below, a new type of protease has been identified in

skeletal **muscle** and shown to be part of the UCDEN complex. The new protease, multipain, forms a complex of approximately 1500 kDa. . .

DETD a) by itself degrades ubiquitinated **proteins** in an ATP-dependent process and has little or no activity against typical proteasome substrates, such as N-succinly-Leu-Leu-Val-Tyr-7-amino-4-methyl-coumarin (sLLVT-MCA) and casein;

DETD The new protease has also been shown to degrade nonubiquitinated **protein** (e.g., lysozyme) by an ATP-dependent process, although at a slower rate than it degrades ubiquitinated **protein** (ubiquitinated lysozyme), and to degrade oxidant-damaged hemoglobin by an ATP-independent mechanism. The new protease has been shown to play a critical role in the key cytosolic (nonlysosomal) **protein** degradative pathway and to function synergistically with the proteasome (as a constituent of a complex comparable in size to UCDEN) in the ATP-dependent degradation of ubiquitinated **proteins**. In the large complex, multipain appears to catalyze initial cleavages of ubiquitin-conjugated **proteins**. Taken together, the findings presented herein indicate that multipain is the rate-limiting component in the recognition and degradation of ubiquitin-conjugated **proteins**.

DETD As described in detail in Example 1, the new protease has been obtained from mammalian skeletal **muscle**. Briefly, **muscles** were obtained and processed, as described in Example 1, in order to isolate the fraction which included the activity degrading Ub-**protein** conjugates. The activity-containing fraction was further separated by chromatography into two peaks with Ub-**protein** (Ub-.sup.125 I-lysozyme)-degrading activity. Peak 2 was shown to account for most of the ATP-stimulated breakdown of ubiquitinated lysozyme and to. . .

DETD . . . Ub-lysozyme. This suggests that a single type of active site is involved in the hydrolysis of these different types of **proteins** .

DETD Assessment of whether ubiquitinated and non-ubiquitinated **proteins** are bound to the same site on the new protease was carried out (Example 1). Results failed to demonstrate competition. . . degradation of Ub-.sup.125 I-lysozyme). This suggests that the new protease has specific binding domains which recognize both ubiquitinated and nonubiquitinated **protein** substrates.

DETD . . . similar or identical to the 1500 kDa Ub-conjugate degrading enzyme, or 26s proteolytic complex UCDEN, isolated previously from reticulocytes and **muscle**. These structures are of similar sizes, are labile, and are activated by the same nucleotides. They degrade the same substrates. . .

DETD . . . complex. The findings described herein also show that the proteasome and multipain function synergistically in the ATP-dependent degradation of ubiquitinated **proteins**. For example, as described in Example 2, when multipain alone degraded Ub-.sup.125 I-lysozyme, the only .sup.125 I product was a. . .

DETD . . . of the proteasome, which inhibits the proteasome's proteolytic activities has been purified from reticulocytes and shown to be an ATP-binding **protein** whose release appears to activate proteolysis. The isolated inhibitor exists as a 250 kDa multimer and is quite labile (at. . . the inhibitor plays a role in the ATP-dependent mechanism of the UCDEN complex. It is possible, for example, that during

protein breakdown, within the 1500 kDa complex ATP hydrolysis leads to functional release of the 40 kDa inhibitor, temporarily allowing proteasome activity, and that ubiquitinated **proteins** trigger this mechanism.

DETD The purified factor has been shown to inhibit hydrolysis by the proteasome of both a fluorogenic tetrapeptide and **protein** substrates, as described in Example 7. When the inhibitor, the proteasome and partially purified CF-1 were mixed in the presence. . .

DETD . . . the physiological roles of the soluble ATP-Ub-dependent pathway, which is generally believed to be a constitutive process (often termed "basal **protein** breakdown") and to be primarily responsible for the elimination of abnormal or short-lived regulatory polypeptides. As shown herein for the. . . mass and negative nitrogen balance characteristically seen in many disease states or conditions is the result of accelerated or excessive **protein** degradation carried out via this pathway. The **muscle** wasting which occurs upon denervation, fasting, fever or metabolic acidosis is due mainly to this accelerated **protein** breakdown. Now that the responsible pathway and key constituents (e.g., multipain and a natural proteasome regulator) have been identified, it is possible to reduce or abolish the accelerated **protein** breakdown and, thus, the loss of body mass and the negative nitrogen balance. Multiple steps in the ATP-Ub-dependent pathway may be affected in **muscle** by fasting and denervation, but one clear point of regulation is the rate of production of Ub mRNA, as shown in Example 6. In addition, increased conjugation of **muscle proteins** to ubiquitin has been shown under these conditions.

DETD . . . can serve as the basis for effective methods for reducing this proteolytic process and, thus, combatting negative nitrogen balance and **muscle** wasting in such conditions as cachexia associated with diseases including various types of cancer and AIDS, febrile infection, denervation atrophy. . . inhibition of the ATP-ubiquitin-dependent pathway is an approach to treatment. This results in reduction (total or partial) of the accelerated **protein** breakdown which occurs in numerous physiological and pathological states, but does not affect normal degradative processes carried out via this. . .

DETD . . . play a critical role in the cytosolic proteolytic pathway which has been shown to be activated in various forms of **muscle** wasting. The availability of purified multipain of the present invention makes it possible to define the enzyme's active site or. . .

DETD . . . a key participant and whose activation, as shown for the first time herein, is responsible for most of the increased **protein** degradation which occurs in skeletal **muscle** during fasting, denervation and infection. Inhibitors can be produced which interact specifically with a particular subunit or polypeptide which is. . .

DETD . . . is intended to include DNA encoding the purified multipain obtained as described, DNA encoding a multipain subunit, DNA encoding a **protein** or polypeptide which has substantially the same activity and functional characteristics as those of the purified multipain obtained as described. . .

DETD . . . described herein, Ub mRNA levels increase (i.e., the polyUb gene is specifically induced) under conditions where there is enhanced ATP-dependent **protein** degradation (e.g., atrophying

muscle, fasting). These levels return to normal when the enhanced degradation is reversed (e.g., by refeeding). An appropriate oligonucleotide probe can. . . and determine whether it is present in greater than normal quantities. This can be used as an indicator of accelerated **protein** degradation.

DETD . . . inhibitor to interfere with activity of the protease. For example, a potential inhibitor can be combined with multipain, a ubiquitinated **protein** substrate (e.g., ubiquitinated lysozyme), ATP and Mg.sup.2+, under conditions appropriate for the protease to degrade the ubiquitin-**protein** conjugate. A control which includes the same components except for the potential inhibitor is used for comparative purposes. Inhibitors are. . .

DETD . . . inhibitors, as well as proteasome inhibitors and UCDEN inhibitors, can be used to reduce (totally or partially) the nonlysosomal ATP-dependent **protein** degradation shown to be responsible for most of the increased **protein** degradation which occurs during fasting, denervation or disuse (inactivity), steroid therapy, febrile infection and other conditions. As described herein, cystatin. . .

DETD . . . be necessary to determine whether any inhibitors found to be effective against the 1500 kDa proteolytic complex can selectively inhibit **protein** breakdown in intact cells. This can be done as follows: First, crude extracts of **muscle** will be used to test the inhibitor's ability to block the entire ATP-ubiquitin-dependent pathway. Such studies can use model radioactive substrates as well as endogenous cell **proteins**, whose degradation can be easily followed by measuring the appearance of free tyrosine. I. C. Kettelhut, et al., Diabetes/Metab., Rev. 4:751-772 (1988); M. Tischler, et al., J. Biol. Chem. 257:1613-1621 (1982). Promising agents are then tested on intact rat **muscles** and cultured cells, in order to evaluate their efficacy against the intracellular proteolysis, their ability to permeate mammalian cells, and. . .

DETD . . . for their ability to inhibit the ATP-ubiquitin-dependent degradative process is to do so in cultured cells in which a short-lived **protein** whose degradation is ubiquitin-dependent is produced. Inhibition of the process leads to accumulation of the **protein** in the cytosol. The extent to which the **protein** accumulates in the cytosol can be determined, using known methods. For example, a potential inhibitor of the process can be. . . potential inhibitor being tested. Cultured cells, such as COS cells, which are stably transformed with a gene encoding a short-lived **protein** whose degradation is ubiquitin-dependent (e.g., a short-lived enzyme, such as a mutant .beta.-galactosidase with an abnormal amino terminus which marks. . .

DETD If a substance which blocks **protein** synthesis is added to such cells, the enzymatic activity and antigen (**protein**) disappear equally rapidly, making it possible to confirm the potential inhibitor's actions on proteolysis. Measurement of cell growth, ATP content and **protein** synthesis in such cells makes it possible to identify (and avoid) highly toxic substances, which is useful because any agent. . .

DETD . . . would also be informative to use pulse-chase isotopic methods to follow the rates of breakdown of endogenous short-lived and long-lived **proteins**, especially long-lived **proteins**, especially ones known to be degraded by the ubiquitin dependent pathway

(e.g., the oncogene products myc or fos).

DETD Any effective inhibitors are then tested in vitro in incubated rats. In such experiments, the soleus or extensor digitorum longus **muscles** from one leg can be incubated with an inhibitor, while the contralateral, identical **muscle** serves as a control. The great advantage of such approaches is that they are highly sensitive, inexpensive, and do not. . . al., J. Biol. Chem., 265:8550-8557 (1990). With experience, it is easy,with six animals to demonstrate statistically significant changes in overall **protein** breakdown or synthesis as small as 10-15%. It can be calculated from the average turnover time of **muscle proteins** that even changes of this magnitude in proteolysis could be of therapeutic benefit; if maintained for 2 weeks, a 15% reduction in proteolysis by itself should lead to at least a doubling of mass of a denervated **muscle**. Also of interest will be to follow the effects of the inhibitor on breakdown of myofibrillar **proteins**, which constitutes 60% of the **muscle** mass, and represent the major **protein** reserve in the organism. These **proteins** are lost differentially upon denervation or fasting. K. Furuno, et al., J. Biol. Chem., 265:8550-8557 (1990). The degradation of myofibrillar components can be followed specifically by measuring 3-methylhistidine release from **muscle proteins**, which is a specific assay for breakdown of actin. K. Furuno, et al., J. Biol. Chem., 265:8550-8557 (1990); B. B. Lowell, et al., Biochem. J., 234 (1986). It will be of particular importance to carry out such studies with **muscles** undergoing denervation (disuse) atrophy or ones from fasted or endotoxin-treated (febrile) animals. In such tissues, overall **protein** breakdown is enhanced, and thus they closely mimic the human disease, but can be studied under well-defined in vitro conditions.. . .

DETD Inhibition of the **protein** degradative process will be useful in a wide variety of conditions in which **muscle** wasting occurs and exacerbates the effects of the underlying condition, further weakening the affected individual. Such conditions include cancer, AIDS, **muscle** wasting after surgery or injury (due to immobilization of the individual or a limb), infection, cachexia due to any cause,. . .

DETD . . . administered to counter weight loss which occurs in animals or to act as growth promoters. Since they act to inhibit **protein** breakdown they should promote net **protein** accumulation and make **protein** synthesis more efficient in growth promotion. For example, they can be administered to animals in order to avoid the epidemic loss of **muscle** mass (net **protein** degradation), referred to as shipping fever, that generally occurs when sheep or cattle are immobilized or confined, such as during. . .

DETD . . . another multipain inhibitor or an inhibitor of another pathway (e.g., a lysosomal or Ca.sup.2+ -dependent pathway) responsible for loss of **muscle** mass.

DETD . . . .sup..cndot. O.sub.2 radicals generated by .sup.60 Co irradiation at a concentration of 50 nmol of oxygen radicals per nmol of **protein**. Davies, K. J. A. J. Biol. Chem., 262:9895-9901 (1987). Casein and lysozyme were radiolabelled with .sup.14 C-formaldehyde and .sup.125 I,. . .

DETD New Zealand white rabbits (4-5 kg) were killed by asphyxiation with CO.sub.2, and the psoas **muscles** were rapidly excised. The **muscles** were trimmed of fat and connective tissue, and then ground on a prechilled meat grinder. Approximately 250 g of

muscle (wet weight) were suspended in ice-cold buffer (3 ml/per g of tissue) containing 20 mM TRIS-HCl (pH 8.0), 1 mM. . .

DETD . . . column equilibrated in 20 mM TRIS-HCI (pH 7.0) and 1 mM DDT (buffer A). The column was washed until no **protein** was detected in the eluate, and the bound **protein** (Fraction II), which contains most of the ATP-dependent proteolytic activity, was eluted with buffer A containing 0.5 M NaCl. The eluted **proteins** (Fraction II)-were submitted to ammonium sulfate fractionation.

DETD In order to remove the free proteasome from other activities, **muscle** fraction II was brought to 38% saturation and stirred for 45 min. The insoluble **proteins** were isolated by centrifugation at 10,000.times.g for 20 min, and the 0-38% pellet was then suspended in 20 mM TRIS-HCl. . .

DETD . . . 200 .mu.l containing 50 mM TRIS-HCl (pH 7.8), 10 mM MgCl.sub.2, 1 mM DTT, and 5 .mu.g of the radioactive **proteins**, 0.5 .mu.g of Ub-conjugates, or 0.5 mM of the fluorogenic peptide. For assays of proteolysis, the reaction mixtures contained approximately 15,000 cpm of Ub-lysozyme or labeled **proteins**. Degradation of .sup.125 I-lysozyme, Ub-.sup.125 I-lysozyme, .sup.14 C-casein, .sup.14 C-hemoglobin and OH/O.sub.2.sup.- treated .sup.14 C-hemoglobin were assayed by measuring the. . .

DETD Electrophoresis **Proteins** were analyzed by SDS-PAGE (10% polyacrylamide gels), as described by Laemmli. Laemmli, U.K. Nature (London) 227:680-685 (1970). The gel was. . .

DETD Immunoprecipitations were performed by incubation of anti-proteasome IgG (100 .mu.g) with **protein** A-Sepharose, as previously described. Matthews et al., Proc. Natl. Acad. Scii. USA 86:2597-2601 (1989). Control immunoprecipitations were performed using Hyclone. . . rabbits by T. Edmunds and A. L. Goldberg. Matthews et al., Proc. Natl. Acad. Sci. USA, 86:2597-2601 (1989). For immunoblotting, **proteins** were electrophoresed on a 10% SDS-poly-acrylamide gel. After transferring the **proteins** to nitrocellulose sheets, (Hershko et al., Proc. Natl. Acad. Sci. USA, 77:1783-1786 (1980)) immunoblots were performed as previously described. Hough. . .

DETD . . . Chem. 261:2400-2408 (1986), Hough, R., and Rechsteiner, M. J. Biol. Chem. 261:2391-2399 (1986)) but using liver extracts. Although this ubiquitinated **protein** was degraded only slowly in crude extracts, fraction II (the fraction that binds to DEAE-cellulose and contains the ATP-dependent degradative. . . in the absence of Mg.sup.2+ (and in the presence of 1 mM EDTA) did not stimulate the degradation of Ub-conjugated **proteins**.

DETD TABLE I

PURIFICATION SCHEME FOR THE 500kDA PROTEASE FROM RABBIT SKELETAL **MUSCLE** WHICH DEGRADES UBIQUITINATED LYSOZYME

Fraction	Total **protein** (mg)	Specific activity (cpm/h .times. mg) +ATP	Specific activity (cpm/h .times. mg) -ATP	ATP stimulation (+ATP/-ATP)
Crude extract	17433	82	74	1.1
DE52 eluate (Fraction. . .	1170	779	338	2.3

DETD . . . et al., J. Biol. Chem. 262:2451-2457 (1987), Driscoll, J., and

Goldberg, A. L. J. Biol. Chem. 265:4789-4792 (1990). The pelleted **proteins** were resuspended, dialyzed, and chromatographed on a column using Mono Q-FPLC (Pharmacia). Two peaks with Ub-.sup.125 I-lysozyme-degrading activity were found. . . . megapain complex. However, it is noteworthy that this structure degrades non-ubiquitinated lyxozyme perhaps as readily as it degrades the Ub-conjugated **protein**.

DETD . . . in its Mr (600 KDa) and ability to hydrolyze sLLVT-MCA, peak 4 resembles the proteasome, but it did not degrade **proteins** (lysozyme, casein or hemoglobin) for reasons that are uncertain.

DETD Due to difficulties in preparation of large amounts of Ub-conjugated **proteins**, the concentration of ubiquitinated lysozyme used in the standard assays was about 10 times lower than that of free lysozyme. . .

DETD . . . of the new enzyme when assayed against lysozyme, Ub-lysozyme, or oxidant-treated hemoglobin, although these treatments quantitatively precipitated the purified rabbit **muscle** proteasome, as assayed with .sup.14 C-casein or sLLVT-MCA (Table II). (These various proteasome activities are not directly inhibited by the antibodies, but in these experiments, these activities were removed together by precipitations with **protein** A-Sepharose). The absence of cross-reactivity between these two multimeric proteases was confirmed by Western blot, where these monoclonal or polyclonal. . . .

DETD Table III presents the effects of nucleotides on the degradation of Ub-.sup.125 -lysozyme by the new activity from skeletal **muscle** . In these assays, the active peak from the Suparose 6 chromatography was incubated with Ub-.sup.125 I-lysozyme at 37.degree. C. for. . .

DETD TABLE III

EFFECT OF NUCLEOTIDES ON THE DEGRADATION OF Ub-.sup.125 I-LYSOZYME BY THE NEW ACTIVITY FROM SKELETAL **MUSCLE**

Compound	Relative activity (%)
None	100
ATP	743
ADP	113
AMP	130
AMP-PNP	90
ATP-.gamma.-S	103
CTP	373
GTP	435
UTP. . .	

DETD . . . requirement for ATP could also be satisfied in part by CTP or GTP, which caused approximately a 4-fold stimulation of **protein** breakdown (Table III). This nucleotide requirement thus resembles prior findings for the nucleotide specificity for Ub-conjugate degradation by the 1500. . . . the proteasome, in which any nucleotide triphosphate, including nonhydrolyzable analogs, could activate hydrolysis of peptide substrates, but the stimulation of **protein** breakdown was only seen with ATP.

DETD . . . to be physiological, and this K.sub.m is consistent with earlier observations on cultured cells, where depletion of cellular ATP blocks **protein** breakdown only when ATP levels are reduced drastically (>75%).

DETD TABLE IV

EFFECT OF INHIBITORS ON DIFFERENT ACTIVITIES
OF THE NEW PROTEASE AND ON THE PROTEASOME
FROM SKELETAL **MUSCLE**

Addition	Relative activity (%) NEW PROTEASE Ub-lysozyme	lysozyme	OH/O.sub.2 - treated hemoglobin	PROTEASOME SLLVT-MCA
None	100	100	100	100
DFP	96. . .			

DETD The new protease was incubated at 37.degree. C. for 1 h with the **protein** substrates and 2 mM ATP. The proteasome obtained by Superose 6 chromatography was incubated with sLLVT-MCA. Mixtures were preincubated for. . .

DETD . . . A similar effect of cystatin was previously reported for the ATP+Ub-dependent proteolysis against the very large UCDEN complex from rabbit **muscle**. The inhibition by Stefin A is physiologically interesting, since homologous **protein** inhibitors are present in many mammalian tissues. At similar concentrations, cystatin B showed a 55% inhibition, and no significant effect. . .

DETD . . . seems most likely that a single type of active site is involved in the hydrolysis of these different types of **proteins**.

DETD . . . cystatin and other inhibitors to reduce the degradation of Ub-conjugates correlated with their ability to inhibit breakdown of the other **proteins**. The simplest interpretation of these data would be that all three substrates are degraded by a single active site or. . .

DETD To test if ubiquitinated and nonubiquitinated **proteins** were bound to the same site, the purified enzyme was incubated for 1h at 37.degree. C. in the presence of. . . 0.5 .mu.g of lysozyme), even though the nonlabelled lysozyme and oxidized hemoglobin decreased linearly the breakdown of the homologous radioactive **proteins**. In addition, no competition was detected between lysozyme and oxidant-treated hemoglobin at these concentrations. This failure to demonstrate competition between those 3 substrates suggests that the protease has specific binding domains that recognize these different **protein** substrates and also that Ub-lysozyme breakdown does not involve generation of free lysozyme.

DETD . . . in the presence of ATP. To test this hypothesis, approximately equal amounts of multipain and extensively purified proteasome isolated from **muscle** were incubated at 37.degree. C., with or without Mg.sup.2+ -ATP. Active peaks (1 mg **protein** each) obtained after Superose 6 gel filtration were incubated together in the presence of 1 mM Mg ATP for 30. . .

DETD Fractionation of **muscle** extracts- The psoas **muscles** were excised from New Zealand White (4-5 kg) male rabbits (Millbrook Farms, Mass.), and post-mitochondrial extracts were prepared and fractionated on DEAE-cellulose, as described in Example 1. The **proteins** absorbed to DEAE-cellulose and eluted with 0.5 M NaCl (Fraction II) were subjected to (NH4)2SO4 fractionation in order to separate. . .

DETD . . . 200 .mu.l containing 50 mM TRIS-HCI (pH 7.8), 10 mM MgCl.sub.2, 1 mM DTT, and 5 .mu.g of the radioactive **proteins**, 0.5 .mu.g of .sup.125 I-lysozyme conjugates or 0.5 mM of the fluorogenic peptide, succinyl-Leu-Leu-Val-Try-7-amido-4-methylcoumarin (sLLVTA-MCA). The amount of Ub-conjugates was. . .

DETD **Protein** was assayed by the method of Bradford. (Bradford, M.

M., Anal. Biochem. 72:248-254 (1976)). **Proteins** were analyzed by SDS-PAGE (10% polyacrylamide) using the method of Laemmli (Pickart, C. M. et al., Arch. Biochem. Biophys. 272:114-121. . . .

DETD In addition to degrading Ub-lysozyme, the 1500 kDa complex degraded a variety of unconjugated **protein** substrates, as do multipain and the proteasome (Table V, FIG. 7).

DETD . . . OH/O.sub.2.sup.- -treated sLLVT-MCA Activity Hemoglobin Hemoglobin (units)

Proteasome	1	30	133
Multipain	0.4	7	5
Complex	0.7	29	162

*All **protein** substrates were at 25 .mu.g/ml except Ub.sup.125 Ilysozyme, which was present at 2.5 .mu.g/ml. (For .sup.125 IUb-lysozyme, this concentration refers. . . .

DETD . . . by 65%. A similar inhibition by cystatin of ATP-Ub-dependent proteolysis was previously reported for the UCDEN complex isolated from rabbit **muscle**. Fagan, J. M., et al., Biochem. J., 243:335-343 (1987). Other inhibitors of thiol proteases, like leupeptin or E64, did not. . . .

DETD . . . ATP could also be satisfied in part by CTP or GTP, which caused approximately a 3- to 4-fold stimulation of **protein** breakdown (Table VII). The nucleotide-specificity of the complex resembles prior findings for the nucleotide-specificity for UB-conjugate degradation by reticulocyte extracts,. . . . with the activation of the isolated proteasome, which only occurs with ATP and thus probably involves a distinct nucleotide binding **protein**.

DETD . . . ATP would appear to be physiologiclly relevant. Furthermore, this Km is consistent with earlier observations on the energy requirement for **protein** breakdown in intact fibroblasts (Gronostajski, R., Pardee, A. B., and Goldberg, A. L., J. Biol. Chem., 260:3344-3349 (1985)), in which nonlysosomal **protein** breakdown fell only when ATP cellular levels were reduced by more than 70% (i.e., from about 3 mM to below. . . .

DETD . . . of the three components (CF-2) of the 1500 kDa complex. Recently an ATPase which corresponds to one of the proteasome-associated **proteins** of 95-105 kDa and which may regulate proteasome activity within the complex has been purified. Multipain and the larger complex. . . . for cystatin-sensitive proteolytic activity. Thus, in addition to degrading ubiquitinated lysozyme to small peptides, isolated multipain rapidly disassembles multiple ubiquitinated **protein**, releasing free ubiquitin and **protein**.

DETD Within the 1500 kDa complex, the proteasome and multipain appear to act synergistically in the breakdown of Ub-conjugated **proteins**. Both the rate and extent of conjugate degradation were greater with the complex than with equal roles of multipain alone.. . . . to function in an integrated, perhaps processive, manner. The complex yields short oligopeptides, although in vivo and in reticulocyte extracts, **proteins** are digested all the way to free amino acids. Presumably other exopeptidases catalyze the completion of this hydrolytic pathway.

DETD . . . If the lag phase also occurs in-vivo, it may mean that if a multipain molecule by itself binds a ubiquitin-conjugate, **protein** degradation proceeds very slowly until multipain also

interacts with a proteasome and forms the larger, more active degradative complex.

DETD Demonstration of Activation of the Cytosolic ATP-Dependent Proteolytic Pathway in Atrophy of Skeletal **Muscle** Upon Denervation (Disuse)

DETD . . . described in Examples 3 and 4, activation of the nonlysosomal (cytosolic) ATP-independent proteolytic pathway has been demonstrated in striated (skeletal) **muscle** during denervation atrophy and fasting and has been shown to be responsible for most of the increased **protein** degradation which occurs in both states.

DETD **Muscle** Incubations

DETD . . . young (60-80 g) male Charles River rats, which were given free access to water and Purina Lab Chow. The soleus **muscle** was denervated as described previously (Furuno K. et al., J. Biol. Chem. 265:8550-8557 (1990)) and sham-operated rats used as controls.. . . the sciatic nerve or after withdrawal of food, the rats were killed and the soleus or extensor digitorum longus (EDL) **muscles** were dissected and incubated in vitro, as described previously. Furuno K. et al., J. Biol. Chem. 265:8550-8557 (1990); Baracos, V.. . . al., Am. J. Physiol. 251:C588-596 and Kettlehut, I. C. Am. J. Physiol., in press (1991). After a 1 hour preincubation, **muscles** were transferred to fresh medium, and tyrosine release measured after 2 hours. The Ca.sup.2+ -free Krebs-Ringer bicarbonate buffer used in. . . .mu.g/ml insulin, 0.17 mM leucine, 0.1 mM isoleucine, 0.2 mM valine, 10 .mu.M methylamine, and 50 .mu.M E-64. To deplete **muscles** of ATP, they were incubated with dinitrophenol (at 0.1 and 0.5 mM) and 2 deoxyglucose (5 mM) after removal of. . .

DETD To measure overall **protein** breakdown, the release of tyrosine from cell **proteins** was followed under conditions where **protein** synthesis was blocked. The accumulation of 3-methylhistidine was measured to follow the breakdown of myofibrillar **proteins**; 3-methylhistidine is a specific constituent of actin and myosin Goodman, M. N. Biochem. J. 241:121-127 (1987) and Lowell, B. B.. . .

DETD The ATP content of the **muscles** was determined after preincubation with or without metabolic inhibitors, as described previously. Gronostajski, R. et al., J. Biol. Chem., 260:3344-3349. . .

DETD Measurement of ATP-depletion on Proteolysis in Skeletal **Muscle**

DETD A simple experimental approach to measuring reliably the ATP-dependent system in intact **muscle** in vitro has been developed.

DETD Despite the fact that **muscle** extracts contain the ATP-Ub-dependent system, Matthews, W., et al., Proc. Natl. Acad. Sci. USA, 86:2597-2601 (1989) and Fagan, J. M.,. . . Fagan, J., J. Biol. Chem., 264:17868-17872 (1989), efforts have repeatedly failed to demonstrate a fall in proteolysis upon depleting intact **muscles** of ATP by using metabolic inhibitors. Goodman, M. N., Biochem. J., 241:121-127 (1987). In other cells studied, including fibroblasts, hepatocytes,. . . A. L., and St. Johh, A., Ann. Rev. Biochem., 45:747-803 (1976), agents that block ATP production were found to reduce **protein** breakdown by 50-90%. However, when rat leg **muscles** were incubated in normal media (containing Ca.sup.2+) with cycloheximide, dinitrophenol (DNP), and 2-deoxyglucose, **muscle** ATP content decreased by over 90%, yet overall proteolysis increased by 80-200%. Fulks, R., et al., J. Biol. Chem., 250:290-298 (1975). Both the dark soleus and the pale EDL **muscles** showed a similar activation of proteolysis upon

ATP-depletion, as did soleus **muscles** following denervation or fasting of the animals for 2 days. This rise in proteolysis was seen even when the **muscles** were incubated under conditions that reduce net **protein** breakdown (i.e., incubation under tension with insulin and amino acids present). Baracos, V. E., and Goldberg, A. L., Am. J. Physiol., 251:C588-596 (1986). Under these conditions, the **muscles** developed rigor, as is typical upon ATP-depletion. A variety of evidence (see below) indicated that this anomalous activation of proteolysis was because ATP depletion in **muscle** leads to Ca.sup.2+ entry into the cytosol and activation of Ca.sup.2+ dependent proteases, and that the resulting stimulation of overall. . .

DETD TABLE IX

EFFECT OF INHIBITORS OF DIFFERENT CELL PROTEASES AND ATP PRODUCTION ON BREAKDOWN OF MYOFIBRILLAR AND TOTAL **PROTEIN** IN DENERVATED SOLEUS

	Total **Proteins**		Myofibrillar **Proteins**	
Pathway Inhibited	Tyrosine Release (pmol/mg/2h)	(%)	3-Methylhistidine Release (pmol/mg/2h)	(%)
None	328 .+-. 10	100	5.11 .+-. 0.21	100
Lysosomal Pathway +Ca.sup.2+ Dependent + Lysosomal	330 .+-.. . . .+-. 14*	34	2.24 .+-. 0.17*	44

Values are the means .+-. SEM for 5 **muscles** three days after section of the sciatic nerve. Significant difference, *p < 0.01. **Protein** breakdown measured in **muscles** at resting length in Ca.sup.2+free KrebsRinger bicarbonate buffer containing insulin and amino acids. Methylamine (10 .mu.M) is an inhibitor of. . .

DETD Conditions for Measuring ATP-dependent Proteolysis in Incubated **Muscles**

DETD . . . measure the ATP-dependent process, it was necessary to prevent the activation of Ca.sup.2+ -dependent proteases upon ATP-depletion (see above). The **muscles** were therefore maintained at resting length (Baracos, V. E., and Goldberg, A. L., Am. J. Physiol., 251:C588-596 (1986)), in Ca.sup.2+. . . al., Agric. Biol. Chem., 42:523-528 (1978). Prior studies showed that these conditions block the activation of proteolysis in anoxic (shortened) **muscles** (Baracos, V. E. and A. L. Goldberg, Am. J. Physiol., 251:C588-596 (1986); and Kettelhut, I. C. et al., Am. J.. . . al., Am. J. Physiol., 13:E702-71- (1986)). As described previously, in this medium inhibitors of ATP production were found to reduce **protein** breakdown in **muscle** (FIG. 9), as they do in other cells. Gronostajski, R., et al., J. Biol. Chem., 260:3344-3349 (1985) and Goldberg, A. L., and St. John, A., Ann. Rev. Biochem., 45:747-803 (1976). To prevent lysosomal **protein** breakdown in these **muscles** (Furuno K., and Goldberg, A. L., Biochem. J., 237:859-864 (1986); Zeman, R. J. et al., J. Biol. Chem., 260:13619-13624

(1985). . . . S. J. Cell. Biol., 90:665-669 (1981). In addition, the E-64c inactivates lysosomal thiol proteases (cathepsins B,H, and L) in intact **muscles**, Baracos, V. E., et al., Am. J. Physiol., 13:E702-710 (1986). These incubation conditions do not affect the levels of ATP or creatine phosphate in the tissues or the rates of **protein** synthesis. Baracos, V. E., et al., Am. J. Physiol. 251:C588-596 and Kettlehut, I. C. Am. J. Physiol., in press (1991).

DETD Even though lysosomal and Ca.sup.2+ -dependent proteolytic systems were blocked, the **muscles** showed linear rates of **protein** breakdown (FIG. 10). These rates were similar to those in **muscles** maintained in complete medium lacking the inhibitors. Baracos, V. E., et al., Am. J. Physiol. 251:C588-596 (1986); Kettlehut, I. C.. . . . This finding agrees with prior studies showing that lysosomal and Ca.sup.2+ -dependent processes make a very minor contribution to "basal" **protein** breakdown. Rechsteiner, M., Ann. Rev. Cell. Biol., 3:1-30 (1987); Dice, J. G., FASEB J., 1:349-356 (1987); Gronostajski, R., et al.,. . . . 260:13619-13624 (1985) and Baracos, V. E., and Goldberg, A. L., Am. J. Physiol., 251:C588-596 (1986). When normal soleus or EDL **muscles** in this medium were depleted of up to 96% of their ATP (with dinitrophenol and 2-deoxyglucose), there was a 50-70% reduction in **protein** degradation (FIG. 10), which resembles the fraction of **protein** breakdown that is ATP-dependent in fibroblasts. Gronostajski, R., et al., J. Biol. Chem., 260:3344-3349 (1985). To quantitate this ATP-dependent component, the **muscle** of one limb was depleted of ATP, while the contralateral **muscle** served as a control. The rate of **protein** degradation in the two limbs were compared. The net decrease in overall **protein** breakdown comprises the ATP-dependent component and could thus be measured highly reproducibly in **muscles** in different physiological states (FIGS. 9 and 10). Kettelhut, I. C., et al., Diabetes/Metabolism Reviews, 4:751-772 (1988); Han, H. Q.,. . .

DETD To deplete **muscles** of ATP, they were preincubated for 1 hour with 2,4-dinitrophenol (DNP) and 2-deoxyglucose to block both oxidative phosphorylation and glycolysis.. . . . (1985)), and hepatocytes, Hershko, A., and Tomkins, G. M., J. Biol. Chem., 246:710-714 (1971), these agents block ATP production and **protein** breakdown reversibly. Neither inhibitor affected the ATP-dependent or energy-independent proteolytic systems in cell-free extracts of **muscle**. Typically, preincubation with DNP (0.1 mM) and 2-deoxyglucose (5 mM) for 1 hour reduced ATP content by >85%, and 0.5 mM DNP with deoxyglucose (5 mM) depleted ATP by >96% in normal **muscles**. These treatments caused similar reductions in ATP content in denervated **muscles** and in **muscles** from fasted animals whose initial ATP stores were also similar to those of control **muscles**. These different concentrations of DNP caused a similar reduction in **protein** breakdown. In these ATP-depleted tissues, the residual (energy-independent) **protein** degradation occurred at linear rates for several hours, and the intracellular pools of tyrosine were similar to those in the contralateral (untreated) **muscles**.

DETD Changes in **Protein** Breakdown during Denervation Atrophy

DETD When the sciatic nerve of a rat is cut, the unused soleus **muscle** on that limb undergoes rapid atrophy, losing about 30% of its weight and **protein** content within 3 days. Furuno K., et al., J. Biol. Chem., 265:8550-8557 (1990) and Goldspink, D. F., Biochem. J., 156:71-80

(1976). During this period, overall **protein** breakdown
increases and by 3 days is 2- to 3-fold greater than in the
contralateral control soleus, Furuno K., et al., J. Biol. Chem.,
265:8550-8557 (1990). A similar rise in overall proteolysis was seen
when the denervated and control **muscles** were incubated in
normal Krebs-Ringer bicarbonate or under conditions which prevent
lysosomal or Ca.sup.2+ -dependent proteolysis, Furuno K., et al.,. .
.
DETD To test whether the ATP-dependent pathway is responsible for the
enhanced **protein** breakdown, the atrophying and control soleus
were depleted of ATP at different times after nerve section, as
described above. Control experiments showed that neither denervation
for
3 days nor fasting affected the **muscle'**s initial ATP content
or the decrease in ATP induced with DNP and. deoxyglucose (Table IX).
However, depletion of cellular ATP caused a much larger net decrease in
proteolysis in the denervated **muscles** than in controls (FIG.
9). For example, in a typical experiment these inhibitors decreased
proteolysis by 53.+-.6 pmol/mg/2h (43%) in. . . the residual rates
of
proteolysis in the denervated and control tissues did not differ (FIG.
9). Thus, in the atrophying **muscles**, a nonlysosomal
ATP-dependent proteolytic process seems to be activated, while no
change
occurs in the residual energy-independent process.
DETD Overall **protein** breakdown in the soleus was enhanced by 1 day
after nerve section and then rose progressively during the next 3. .
.
failure to block completely the ATP-dependent pathway. The rise in the
ATP-requiring process could account for all of the increased
protein breakdown in the denervated **muscle** maintained
in this way (FIG. 9).
DETD Demonstration of Activation of the Cytosolic ATP-Dependent Proteolytic
Pathway in Atrophy of Skeletal **Muscles** in Fasting
DETD **Muscles** of fasting rats were studied to test whether this
degradative process is activated under other physiological conditions
where **muscle protein** breakdown rises. In animals
deprived of food, there is a rapid increase in **muscle**
protein breakdown which appears essential to provide the
organism with amino acids for gluconeogenesis. Li, J. B., and Goldberg,
A. L.,. . . A. L., et al., Federation Proc., 39:31-36 (1980) and
Lowell, B. B., et al., Metabolism, 35:1121-112 (1986). When the EDL
muscles from fasted animals were incubated under conditions that
block lysosomal and Ca.sup.2+ -dependent degradative processes, they
showed a large increase. . . (FIG. 10), in accord with observations
on 3-methyl-histidine production, Lowell, B. B., et al., Metabolism,
35:1121-112 (1986). However, when the **muscles** from the fasted
or fed animals were incubated with metabolic inhibitors to prevent the
ATP-requiring process, these differences in their rates of
protein breakdown were eliminated. Thus, the increase in
muscle proteolysis in fasting seems to be due to an enhancement
of an energy-requiring nonlysosomal process.
DETD . . . evident 1 day after removal of food and could account for all
of the increased proteolysis seen in the EDL **muscle** under
these incubation conditions (FIG. 12). In fasting, the enhancement of
overall proteolysis is greater in the pale **muscles**, such as
the EDL, than in the dark soleus. Li, J. B., and Goldberg, A. L., Am.
J.
Physiol., 231:441-448 (1976). Accordingly, the soleus **muscle**
showed a similar, but a smaller, rise in the ATP-dependent process. On

the average, the rise in proteolysis in the. . .

DETD Upon refeeding the rats, **protein** breakdown in the EDL decreased back to basal levels within 1 day (FIG. 10). Again, this response was due to. . .

DETD One of the major features of denervation atrophy is differential loss of

myofibrillar **proteins**, but the system responsible for their accelerated degradation has not been identified. Furuno K., et al., J. Biol. Chem., 265:8550-8557 (1990). The breakdown of these **proteins** can be followed by measuring 3-methyl-histidine production, which is a specific constituent of actin, and in certain **muscles** of myosin. Goodman, M. N., Biochem. J, 241:121-127 (1987) and Lowell, B. B., et al., Metabolism, 35:1121-112 (1986). When these **proteins** are hydrolyzed, this amino acid cannot be reutilized in **protein** synthesis, and thus its appearance as an indication of myofibrillar **protein** breakdown. Goodman, M. N., Biochem. J, 241:121-127 (1987) and Lowell, B. B., et al., Metabolism, 35:1121-112 (1986). The increased production. . . lysosomal and Ca.sup.2+ -dependent proteolysis. Furuno K., et al., J. Biol. Chem., 265:8550-8557 (1990). These findings and those for overall **protein** breakdown (FIG. 11) indicate that enhancement of a nonlysosomal ATP-dependent process is primarily responsible for the **muscle** atrophy.

DETD Measurement of Proteolysis in **Muscle** Extracts

DETD Psoas **muscles** from fed and fasted rabbits were used to obtain sufficient material for assay of the ATP-dependent system in cell-free extracts.. . . 20% (similar to that seen in rats deprived of food for

1 day). The animals were anesthesized, and their psoas **muscles** dissected and homogenized as described previously. Fagan, J. M., et al.,

J. Biol. Chem., 261: 5705-5713 (1986).

DETD After centrifugation at 10,000.times.g and then at 100,000.times.g, the **muscle** extracts were fractionated on DE52 cellulose to remove ubiquitin and most cell **proteins**, as described previously. Han, H. Q. et al., Federation Proc, 2:A564 (1988) and Waxman, L., et al., J. Biol. Chem.,. . . containing Tris (50 mM, pH7.8), dithiothreitol (1 mM), and 20% glycerol, and concentrated before assay of activity. Degradation of endogenous **muscle proteins** was assayed by measuring the production of free tyrosine, which was determined fluorometrically after precipitation of **proteins** with trichloroacetic acid. Tischler, M. et al., J. Biol, Chem., 257:1613-1621 (1982) and Fulks, R., et al., J. Biol. Chem.,. . .

DETD . . . the soluble ATP-requiring proteolytic system which involves ubiquitin is activated during fasting or denervation atrophy. However, such measurements on intact **muscles** cannot distinguish other possible changes in these **catabolic** states. Therefore, soluble cell-free extracts of **muscles** from fed and fasted rabbits were used in order to test whether the increased proteolysis in fasting is due to activation of the ATP-Ub-dependent system. Cell-free preparations

showing ATP-Ub-dependent proteolysis have been described in extracts of rabbit **muscles**. Fagan, J. M., et al., Biochem. J., 243:335-343 (1987). The proteolytic system from rabbit **muscles** was partially purified by high-speed centrifugation and ultracentrifugation to remove myofibrils and membranous components, and then it was subjected to DEAE chromatography to remove most (>90%) of the soluble **proteins**, including free ubiquitin. The resulting fraction contains all the enzymes for Ub-conjugation and hydrolysis of Ub-**protein** conjugates, Herskho, A., J. Biol. Chem., 263:15237-15240

(1988); Rechsteiner, M., Ann. Rev. Cell Biol., 3:1-30 (1987); Waxman, L., et al.,. . .

DETD In these extracts, the hydrolysis of endogenous **proteins** (shown by tyrosine production) increased 5- to 9-fold upon addition of ATP and even further upon addition of ATP with. . .

DETD TABLE X

EFFECTS OF FASTING OF RABBITS ON ATP-UBIQUITIN-ACTIVATED PROTEOLYSIS IN EXTRACTS OF PSOAS **MUSCLE**

Condition	No addition	+ATP	+ATP + Ub
Hydrolysis of Endogenous **Proteins** (nmol try released/2 hr)			
Fed	0.6 .+-. 0.1	5.7 .+-. 0.9	9.2 .+-. 1.8
Fasted	2.0 .+-. 0.1	10.8 .+-. 2.1. . .	

These assays were performed on partially purified proteolytic fractions ("Fractions II") as further described in the Example. Breakdown of endogenous **proteins** (tyrosine production) was measured for 2 hours at 37.degree. C. with 5 mg of Fraction II **protein**.

Degradation of .sup.14 CCasein was assayed at 37.degree. C. for 1 hour with 400 .mu.g of #Fraction II **protein** and 20 .mu.g .sup.14 Ccasein.

Assays were performed in Tris (50 mM, pH 7.8), dithiothreitol (1 mM), and MgCl (10. . .

DETD To further test for an activation of the ATP-dependent degradative system, rather than an alteration in the endogenous cell **proteins** which served as substrates, .sup.14 C-methyl-casein was used as a substrate (Table X). This **protein** is also degraded rapidly by ATP-independent enzymes, and this ATP-independent process appeared to increase upon fasting (although this trend did. . .

store large amounts of food in their gastrointestinal tract. However, no such increase in proteolysis was seen in extracts of **muscles** from rabbits deprived of food for shorter periods than 6 days, at which time they showed no weight loss and. . . substrates clearly indicate an increased capacity of the ATP-dependent degradative system in fasting, as suggested by the measurements on incubated **muscles** (FIG. 10).

DETD Further Evidence for Activation of the ATP-Ubiquitin-Dependent Process in Various **Catabolic** States

DETD Activation of the ATP-ubiquitin-dependent proteolytic process was shown to be responsible for most of the increased **protein** degradation in skeletal **muscle** during denervation atrophy, fasting and febrile infection, as described below. In addition, levels of polyubiquitin mRNA and mRNA for proteosome units are shown to increase in skeletal **muscle** during denervation atrophy, fasting and febrile infection, as shown below. Similar data have been obtained in rats with metabolic acidosis (induced by injection with **ammonium chloride**) or suffering with cancer cachexia (induced by a transplantable hepatoma growing in ascites).

DETD **Muscle** preparations

DETD . . . Lab chow and water "ad libitum". All treatments were carried out as described in Example 3. To denervate the soleus **muscles** of one hind limb, the sciatic nerve was cut about 1 cm above the popliteal fossa, while the animals were. . . J. Biol. Chem.,

265:8550-8552 (1990). In all cases the animals were killed by cervical dislocation and the EDL and soleus **muscles** were dissected as described in the previous examples.

DETD Total RNA from **muscle** was isolated by the guanidinium isothiocyanate/CaCl method, and electrophoresis of RNA was performed in 1% agarose gels containing 0.2M formaldehyde.. . . .

DETD . . . dot blot analysis, four different concentrations (2-fold dilutions from 1.5 .mu.g) of total denatured RNA from the soleus or EDL **muscles** were spotted on Gene Screen membranes. The amount of RNA applied to each dot was maintained at 1.5 .mu.g by adding E. coli tRNA (which in the absence or rat **muscle** RNA did not show any hybridization). The hybridization probes were a Ub cDNA fragment (Agell, N. et al., Proc. Natl.. . . dot intensities of the autoradiograms by automated densitometric scanning. The unpaired Student's t-test was used in statistical analyses to compare **muscle** of fed and fasted animals and the paired t-test was used to compare contralateral denervated and control **muscles**.

DETD Measurements of total ubiquitin content (which includes both free Ub and Ub ligated to **proteins**) were carried out using the immunochemical method described by Riley D. A. et al., J. Histochem. Cytochem., 36:621-632 (1988).

DETD To test whether the level of Ub mRNA increases when **muscle** **protein** breakdown rises, the levels of polyUb transcripts in rat **muscles** were determined at different times after food deprivation. As shown in FIG. 11, the soleus contained two transcripts of 2.4. . . in Oxford Surveys on Eukaryotic Genes (Maclean, N., ed.) 4:76-91 (1987)). The levels of both transcripts increased progressively in the **muscles** of fasted animals. The relative levels of Ub mRNA in these tissues of fasting rats were measured by dot-blot analysis. . . (FIG. 12). After 48 hours of food deprivation, the levels of total Ub mRNA in the extensor digitorum longus (EDL) **muscle** showed a 4-fold increase over **muscles** of control animals (FIG. 13, upper panel). The soleus **muscle**, which atrophies less than the EDL in fasting (Li, J. B. and Goldberg, A. L., Am. J. Physiol, 231:441-448 (1976)),. . .

DETD . . . rats were then provided food for 24 hours. By 24 hours of refeeding, the levels of polyUb mRNA in these **muscles** had returned to levels in **muscles** of normal animals. This rise and fall in the amount of polyUb mRNA thus parallels the changes in overall rates of **protein** degradation (FIG. 10) and in the ATP-dependent degradative process (FIG. 10).

DETD . . . mRNA is regulated in a specific manner in fasting, whether the total amount of RNA or of mRNA in these **muscles** may also have changed after food deprivation in a similar way as polyUB mRNA was assessed. The total RNA content. . . amount of mRNA (i.e., poly-A-containing RNA) in the soleus and EDL decreased to approximately 50% of the levels found in **muscles** of fed animals. Total RNA fell from 72.+-.3.5 to 35.+-.1.6 ug/**muscle** and total mRNA (expressed in arbitrary densitometric units) from 2133.+-.376 to 1004.+-.20 units/**muscle** in the soleus during fasting. In the fasted EDL, total RNA decreased from 68.+-.6 to 38.5.+-.1 ug/**muscle** and total mRNA from 710.+-.73 to 413.+-.11 units/**muscle**. The ratio of total mRNA to total RNA, unlike Ub mRNA, thus, did not change significantly during the 48 hours. . . .

DETD Subsequent experiments tested whether the increase in polyUb mRNA in fasting is unique to skeletal **muscle** or whether other rat

tissues show similar responses 2 days after food deprivation. Enhanced proteolysis in fasting has been attributed. . . process. In the heart (ventricle) of fasting rats, a rise in polyUb mRNA occurred similar to that seen in EDL **muscle**. By contrast, no such change was seen in any other tissue tested, including liver, spleen, adipose tissue, brain, testes and kidney. In the liver, kidney, and adipose tissue a marked loss of weight and **protein** occurred on fasting, but as expected neither testes nor brain showed significant weight loss under these conditions. Thus, during fasting, the rise in Ub mRNA appears to be a specific adaptation in striated **muscle** and is not seen in other tissues.

DETD A similar 2- to 3-fold acceleration of the ATP-dependent proteolytic process occurs in **muscle** during denervation atrophy. To test whether in this condition the expression of polyUb genes may also be activated, we analyzed. . . 1 and 3 days following denervation, the levels of polyUb transcripts increased markedly above the levels in the contralateral control **muscle**. Dot blot analysis of the **muscles** revealed a 2 to 3-fold increase in polyUb mRNA content as a proporation of total mRNA following denervation (Table XI). Although the size of Ub mRNA level of control **muscles** did not change during the course of this study, by contrast the total RNA in the denervated soleus decreased by. . .

DETD This increase in mRNA for ubiquitin correlated with accelerated proteolysis in the **muscle**.

DETD TABLE XI

EFFECT OF UNILATERAL DENERVATION OF RAT SOLEUS **MUSCLE** ON THE CONTENT OF PolyUb mRNA, TOTAL RNA AND WEIGHT

me after operation	Control	Denervated	Control	Denervated
mRNA/.mu.g total				
2.4 .+-.. . .				

DETD Ubiquitin Content of the **Muscles**

DETD To determine whether the increase in polyUb mRNA actually resulted in increased production of Ub, the total amount of this **protein** in the **muscles** was quantitated by immunoassay (Table XII). These arrays measured both free Ub and Ub conjugated to cell **proteins**. (Riley, D. A. et al., J. Histochem. Cytochem., 36:621-632 (1988) In EDL **muscles** from animals fasted 2 days, a 63% increase in Ub levels was observed over levels in fed controls. An even larger increase of 91% was seen in the Ub content of soleus **muscles** 2 days after cutting the sciatic nerve. Thus, total Ub content correlated with the increase in ATP-dependent proteolysis and in. . .

DETD The covalent linkage of Ub to cell **proteins** is known to mark them for rapid degradation. Therefore, we also measured the **muscles** content of ubiquitin-**protein** conjugate in normal and denervated **muscle**. As shown in Table XIII, the levels of ubiquitinated **proteins** increased by 158% after denervation for 2 days. A similar increase in ubiquitinated **proteins** was seen upon fasting of the rats (data not shown) and this difference disappeared upon refeeding the animals for one day. These findings further indicate activation of the ubiquitin dependent process in atrophying **muscles**.

DETD In the denervated **muscle** and in fasted animals, there was also found an increase in rate of proteasome synthesis, as indicated by a 2-3. . . and C-9 and in related experiments, a similar increase was seen in mRNA for three other subunits. Thus, the atrophying **muscles** are increasing levels of multiple components of this degradative pathway.

DETD . . . mRNA following denervation, fasting or refeeding occur in parallel with and appear to be linked to the alterations in overall **protein** breakdown and in degradation of myofibrillar **proteins** measured in the incubated **muscles**. The rise in Ub mRNA seen in the atrophying **muscles** appears responsible for their increased Ub content (Table XII), which occurred despite the net loss of total **muscle protein**. Furthermore, the preceding examples demonstrated that these changes in overall proteolysis are due to activation of a nonlysosomal ATP-dependent process and that fasting leads to enhanced ATP-Ub-stimulated proteolysis in soluble extracts of **muscle**.

DETD . . . conclusion that the Ub-dependent proteolytic system is enhanced under these conditions. As described herein, it was also observed that the **muscles** from fasting animals and denervated **muscles** also showed higher levels of Ub-conjugated **proteins** and of mRNA encoding the proteasome, which is essential in the breakdown of such ubiquitinated **proteins**. These results together indicate that the Ub-dependent system in **muscle** is precisely regulated by contractile activity and food intake. The response to fasting requires adrenal steriods (Kettelhut, I. C. et. . .

DETD The changes shown here in Ub mRNA levels parallel exactly the changes in overall **protein** degradation and in the breakdown of myofibrillar **proteins**, both of which were shown in the preceding examples to occur by an ATP-dependent nonlysosomal process. The present data thus suggest a more general role for this system in the degradation of normal **muscle proteins**, including probably the long-lived myofibrillar components.

DETD The polyUb gene seems to be an example of a gene that is specifically induced in atrophying **muscles**. In fasting or denervation atrophy, when **muscle** mass and overall RNA are decreasing, the levels of polyUb mRNA and Ub concentration rose. In contrast, the levels of. . . Ub mRNA levels and Ub production seem to be regulated inversely to total RNA or to mRNA for the Ub-extension **protein** .

DETD . . . physiological interest is the finding that the increase in Ub mRNA (and presumably, therefore, in Ub) is restricted to striated **muscle**. Such changes also occur in the rat heart, which in fasting undergoes considerable weight loss. These findings suggest that ATP-dependent proteolysis also rises in cardiac **muscle** under such conditions, presumably by similar mechanisms as in skeletal **muscle**, although systematic studies have not been reported. The absence of any change in Ub levels in testes or brain was anticipated, since the **protein** content and size of these organs are maintained during a fast. However, it is noteworthy that levels of Ub mRNA. . . relative importance of different proteolytic processes differ between tissues and that the ATP-Ub-dependent pathway is of special significance in striated **muscle**, particularly in **catabolic** states.

DETD TABLE XII

EFFECTS OF DENERVATION AND FASTING ON UBIQUITIN LEVELS IN RAT SKELETAL **MUSCLES**

Muscle	Total **Protein** (mg/**muscle**	Total Ubiquitin (pmol/**muscle**)	(pmol/mg **protein**)
Soleus			
Innervated	3.5 .+-. 0.4	89 .+-. 5	27 .+-. 2
Denervated	2.7 .+-. 0.2	137 .+-. 12	51 .+-. 2
. . . 9	+10 .+-. 2**		
% Change	-32%	+10%	+63%

Values are the means .+-. SEM for extensor digitorum longus (EDL) **muscles** from four fed or fasted animals and for seven paired soleus **muscles** two days following section of one of the sciatic nerves. Significance difference,
*p < 0.05,
**p < 0.01.

DETD . . . AND LEVELS OF UBIQUITIN AND UBIQUITIN-CONJUGATES IN RAT SOLEUS

	ATP-Dependent Proteolysis (pmol tyr/mg/2h	Free Ubiquitin	Ubiquitin Conjugates (pmol Ub/mg **protein**)	Total Ubiquitin
Control	63.0 .+-. 11	17.0 .+-. 1.3	10.0 .+-. 0.7	27 .+-. 1.9
Denervated	201.0 .+-. 17	25.0 .+-. 0.2. . .		

DETD TABLE XV

INJECTIONS OF E. COLI ENDOTOXIN (LPS) RAPIDLY STIMULATE **PROTEIN** BREAKDOWN SIMILARLY IN RAT EXTENSOR DIGITORUM LONGUS **MUSCLE**

Injection Proteolysis (nmol tyrosine/rng/2h)

	Saline	Treated	Difference
LPS	0.214 .+-. 0.013	0.280 .+-. 0.015	+31% P < 0.01

INJECTION OF ENDOTOXIN (LPS) ACTIVATES THE ATP-DEPENDENT PATHWAY OF **PROTEIN** BREAKDOWN IN RAT **MUSCLES**

Addition Proteolysis (nmol tyrosine/mg/2h)

	Saline	Treated	Difference
Non Lysosomal	0.145 .+-. 0.009	0.190 .+-. 0.017	+31%P < 0.05
Proteolysis*			
After ATP	0.094 .+-. 0.004. . .		

DETD Activation of **Protein** Breakdown During Systemic Infections

DETD One other condition where **muscle protein** breakdown increases markedly is during systemic infections of bacterial, viral or parasitic origin. Patients with sepsis, which often follows traumatic injuries, tend to be in marked negative nitrogen balance due mainly to accelerated **muscle** pro breakdown. This response is associated with fever and is part of the body's acute phase response. It can be.

. released by activated macrophages. As shown in Table XV, 6 hours after endotoxin injection, animals were killed and their leg **muscles** studied in vitro. The EDL showed a rapid increase in overall **protein** breakdown. This response was not due to the lysosomal or calcium activated proteases. When the ATP-dependent degradative system was measured, it had increased by 70% and could account for the overall increase of **protein** breakdown in the animals. Treatment of the rats with endotoxin also caused 2-3 fold increase in the levels of polyUb mRNA in these **muscles** within 6-7 hours. This rise in polyUb mRNA which resembles the response seen in fasting or denervation, was not seen in other tissues. Northern analysis of gastrocnemius **muscles**, excised shows after injection of E. coli endotoxin (40 .mu.g/100 g body weight), using cDNA probes of polyUbiquitin genes also showed induction of ubiquitin in RNA (data not shown). These findings thus indicate a common biochemical program in **muscle** leading to enhanced **protein** breakdown in these three **catabolic** states and others, including cancer cachexia as induced in rats carrying Yochida hepatoma in ascities and in rats with metabolic. . .

DETD . . . (Ciechanover, A. et al., Biochem. Biophys. Res. Comm. 81:1100-1105 (1978)). Lysates were then prepared and subjected to DE-52 chromatography. The **protein** eluted with 0.5M KCl (Hershko, A. et al., J. Biol. Chem., 258:8206-8214 (1983)) was concentrated using ammonium sulfate to 80%. . . suspended in 20 mM Tris-HCl (pH 7.6), 1 mM DTT (buffer A). Following extensive dialysis against the same buffer, the **protein** (fraction II) was either stored at -80.degree. C. in 0.5 mM ATP or fractionated further.

DETD . . . for 20 minutes, as described by Ganoth et al. (Ganoth, D. et al., J. Biol. Chem. 263:12412-12419 (1988)). The precipitated **proteins** were collected by centrifugation at 10,000.times.g for 15 minutes. The pellet was resuspended in buffer A and brought again to. . . buffer, the 0-38% pellet was chromatographed on a Mono-Q anion exchange column equilibrated with buffer A containing 10% glycerol. The **protein** was eluted using a 60 ml linear NaCl gradient from 20 to 400 mM. Fractions which inhibited the peptidase activity. . .

DETD . . . ammonium sulfate precipitations. The supernatants were brought to 80% saturation with ammonium sulfate and mixed for 20 minutes. The precipitated **protein** was collected by centrifugation, resuspended in buffer A, and dialized extensively against this buffer. The proteasome was isolated by Mono-Q. . .

DETD . . . was added. Reactions were carried out at 37.degree. C. for 60 minutes with .sup.125 I-lysozyme or 10 minutes with Suc-LLVY-MCA. **Protein** hydrolysis was assayed by measuring production of radioactivity soluble in 10% trichloroacetic acid, and peptide hydrolysis by the release of. . .

DETD These results suggest strongly that the inhibitor corresponds to CF-2 and thus is essential for hydrolysis of Ub-ligated **proteins**. One unusual property of CF-2 is that it is quite labile upon heating to 42.degree. C., but is stabilized by. . .

DETD . . . (1989)). However, a readily apparent band of 40 kDa was evident in this fraction. To further address the question of **proteins** associated with the proteasome, fraction II wsa immunoprecipitated using and anti-proteasome monoclonal antibody and analyzed by SDS-PAGE. Ub-conjugate degrading activity. . .

CLM What is claimed is:

1. A method of screening for an inhibitor of **muscle protein** degradation, said method comprising: a) providing cultured cells in which a **protein** whose degradation is ubiquitin-dependent is produced; b) contacting the cultured cells with a substance to be assessed for its ability to inhibit **muscle protein** degradation, under conditions appropriate for entry of the substance into the cultured cells; c) determining the extent to which the **protein** is present in the cytosol of the cultured cells, wherein accumulation of the **protein** in the cytosol is indicative that the substance inhibits the ATP-ubiquitin-dependent degradative process; and d) identifying a substance that inhibits the ATP-ubiquitin-dependent degradative process as an inhibitor of **muscle protein** degradation.

4. The method of screening for an inhibitor of **muscle protein** degradation as claimed in claim 1, wherein said method further comprises measuring cell growth, ATP content, or **protein** synthesis to identify substances having toxic activity.

5. The method of screening for an inhibitor of **muscle protein** degradation as claimed in claim 1, wherein said **protein** is a short-lived **protein**.

6. The method of screening for an inhibitor of **muscle protein** degradation as claimed in claim 1, wherein said **protein** is a long-lived **protein**.

7. The method of screening for an inhibitor of **muscle protein** degradation as claimed in claim 6, wherein said **protein** is selected from the group consisting of the oncogene product myc and the oncogene product fos.

8. A method of identifying an inhibitor of the ATP-ubiquitin-dependent process, comprising: a) incubating a **muscle**, obtained from an animal afflicted with a **muscle**-wasting condition with a compound to be tested as a potential inhibitor of the ATP-ubiquitin-dependent process, under conditions appropriate for degradation of ubiquinated **proteins**; b) measuring the release of a product of breakdown of **muscle protein** in the presence of the compound; c) comparing the measurement made in (b) with release of the product of breakdown of **muscle protein**, obtained from an animal exhibiting **muscle** wasting, in the absence of the compound; and d) identifying said compound as an inhibitor of the ATP-ubiquitin-dependent process if the presence of said compound results in decreased release of the product of breakdown of **muscle protein**.

9. The method of claim 8 wherein the product of breakdown of **muscle protein** is 3-methylhistidine.

10. The method of identifying an inhibitor of the ATP-ubiquitin-dependent process as claimed in claim 8, wherein said **muscle** is a soleus or extensor digitorum longus **muscle**.

11. The method of identifying an inhibitor of the ATP-ubiquitin-dependent process as claimed in claim 8, wherein said product of breakdown of **muscle protein** is 3-methylhistidine.

12. The method of identifying an inhibitor of the ATP-ubiquitin-dependent process as claimed in claim 8, wherein the **muscle** wasting results from denervation, fasting, febrile infection, or metabolic acidosis.

. . . method of identifying an inhibitor of the ATP-ubiquitin-dependent process in an animal, comprising: a) providing an animal afflicted with a **muscle**-wasting condition; b) administering to the animal a compound to be tested as a potential inhibitor of the ATP-ubiquitin-dependent process; c). . . 3-methylhistidine by the animal; d) comparing the measurement made in (c) with excretion of 3-methylhistidine by an animal subjected to **muscle** wasting without administration of the compound; and e) identifying said compound as an inhibitor of the ATP-ubiquitin-dependent process if the. . .
. . . 14. A method of screening for a potential pharmaceutical agent, said method comprising: a) providing cultured cells in which a **protein** whose degradation is ubiquitin-dependent is produced; b) contacting the cultured cells with a substance to be assessed for its ability. . . process, under conditions appropriate for entry of the substance into the cultured cells; c) determining the extent to which the **protein** is present in the cytosol of the cultured cells, wherein accumulation of the **protein** in the cytosol is indicative of inhibition of the ATP-ubiquitin-dependent degradative process; and d) identifying a substance that inhibits the. . .
. . . potential pharmaceutical agent as claimed in claim 14, wherein said condition is selected from the group consisting of cancer, AIDS, **muscle** wasting after surgery or injury, infection, cachexia, corticosteroid treatment, sepsis, burn, trauma, neuromotor degenerative disease, muscular dystrophy, acidosis, and spinal. . .
17. A method of screening for potential growth promoters, said method comprising: a) providing cultured cells in which a **protein** whose degradation is ubiquitin-dependent is produced; b) contacting the cultured cells with a substance to be assessed for its ability. . . process, under conditions appropriate for entry of the substance into the cultured cells; c) determining the extent to which the **protein** is present in the cytosol of the cultured cells, wherein accumulation of the **protein** in the cytosol is indicative of inhibition of the ATP-ubiquitin-dependent degradative process; and d) identifying a substance that inhibits the. . .

L15 ANSWER 30 OF 109 USPATFULL

AB There are disclosed certain novel compounds identified as benzo-fused lactams which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to increase the stature of those afflicted with a lack of a normal secretion of natural growth hormone. Growth promoting compositions containing such benzo-fused lactams as the active ingredient thereof are also disclosed.

AN 1999:128540 USPATFULL
TI Benzo-fused lactams promote release of growth hormone
IN Wyvratt, Matthew, Mountainside, NJ, United States
Devita, Robert, Westfield, NJ, United States
Bochis, Richard, East Brunswick, NJ, United States
Schoen, William, Edison, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5968924 19991019 <--
AI US 1997-820302 19970318 (8)
RLI Division of Ser. No. US 1995-392961, filed on 18 Apr 1995, now patented,
Pat. No. US 5672596 which is a continuation-in-part of Ser. No. US 1992-936975, filed on 28 Aug 1992, now patented, Pat. No. US 5283241
DT Utility
FS Granted
EXNAM Primary Examiner: Raymond, Richard L.; Assistant Examiner: Kifle, Bruck
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5844
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5968924 19991019 <--
SUMM 1. Increased rate of **protein** synthesis in all cells of the body;
SUMM . . . still further use of the disclosed novel benzo-fused lactam growth hormone secretagogues is in combination with IGF-1 to reverse the **catabolic** effects of nitrogen wasting as described by Kupfer, et al, J. Clin. Invest., 21, 391 (1993).
SUMM . . . These varied uses of growth hormone may be summarized as follows: stimulating growth hormone release in elderly humans; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system; treatment of retardation; acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS. Treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .
DETD . . . at room temperature overnight, then diluted with 1 L of methylene chloride and washed with 500 mL of saturated aqueous **ammonium chloride**, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over magnesium. . .
DETD . . . under nitrogen at -10.degree. C. The suspension was allowed to warm slowly to room temperature over 12 hours then saturated **ammonium chloride** solution (1 L) was added followed by sufficient water (approximately 1 L) to dissolve the precipitate. The solution was extracted. . .
DETD . . . two hours then diluted with 350 mL of methylene chloride. The solution was washed with water (2.times.150 mL), saturated aqueous

ammonium chloride (150 mL), saturated aqueous sodium
bicarbonate (4.times.150 mL) and saturated aqueous sodium chloride (150 mL), dried over sodium sulfate and. . .

DETD . . . at 0.degree. C. for 15 minutes, the reaction mixture was diluted with 400 mL of ethyl acetate and 50% saturated **ammonium chloride**. The mixture was transferred to a separatory funnel and the aqueous layer was separated. The organic layer was washed with. . .

DETD . . . at room temperature for 30 minutes, diluted with 100 mL of ethyl acetate, washed with 25 mL of saturated aqueous **ammonium chloride**, 25 mL of saturated aqueous sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium sulfate,. . .

DETD . . . room temperature, the reaction mixture was diluted with 100 mL of ethyl acetate, washed with 25 mL of saturated aqueous **ammonium chloride**, 25 mL of saturated sodium bicarbonate and 25 mL of brine. The organic layer was removed, dried over magnesium sulfate,. . .

DETD . . . The reaction mixture was stirred for 1 hour then diluted with 150 mL of ethyl acetate, washed with saturated aqueous **ammonium chloride**, saturated aqueous sodium bicarbonate, saturated aqueous sodium chloride, dried over magnesium sulfate and filtered. The solvent was removed under vacuum. . .

DETD . . . ether (4.14 mol, 1.5 eq.). The suspension was allowed to warm slowly to room temperature over 12 hours then saturated **ammonium chloride** solution (1 L) was added followed by sufficient water (approximately 1 L) to dissolve the precipitate. The solution was extracted. . .

DETD . . . C. for 3 hours then cooled to room temperature. The reaction mixture was diluted with 100 mL of saturated aqueous **ammonium chloride**, transferred to a separatory funnel and extracted with ether (3.times.150 mL). The combined ether extracts were washed with saturated aqueous. . .

DETD . . . of 310 mg (0.73 mmol) 2-benzyloxycarbonylamino-2-methyl-N-[7-nitro-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepin-3(R)-yl]propanamide (Step B) in 20 mL of methanol was added 78 mg (1.5 mmol) of **ammonium chloride** followed by 669 mg (10.2 mmol) of zinc dust. The resulting mixture was heated at reflux for four hours. The. . .

CLM What is claimed is:
18. A method for the treatment of the **catabolic** effects of nitrogen wasting which comprises administering to such patient a compound of claim 1 in combination with insulin-like growth. . .
19. A composition for the treatment of the **catabolic** effects of nitrogen wasting which comprises an inert carrier and a compound of claim 1 in combination with insulin-like growth. . .

L15 ANSWER 31 OF 109 USPATFULL

AB The present invention relates to novel organic compounds, to methods for their preparation, to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis.
The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are compounds of formula (I)

(L).sub.n --Ar.sub.1 --R.sub.1 --A (I)

wherein

(L).sub.n, n, Ar.sub.1, R.sub.1 and A are as defined in the application.

AN   1999:117528 USPATFULL
TI   Modulators of molecules with phosphotyrosine recognition units
IN   Andersen, Henrik Sune, Copenhagen, Denmark
        Moller, Niels Peter Hundahl, Copenhagen, Denmark
        Madsen, Peter, Bagsvaerd, Denmark
PA   Novo Nordisk A/S, Bassvaerd, Denmark (non-U.S. corporation)
PI   US 5958957   19990928   <--
AI   US 1997-842801   19970416 (8)
PRAI   DK 1996-46469   19960419
DT   Utility
FS   Granted
EXNAM   Primary Examiner: Richter, Johann; Assistant Examiner: Oswecki, Jane C.
LREP   Zelson, Steve T., Lambiris, Elias J., Rozek, Carol E.
CLMN   Number of Claims: 10
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 2103
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5958957   19990928   <--

AB . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, microorganisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are. . .

SUMM . . . to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of **proteins** or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including **protein** tyrosine phosphatases (PTPases) and **proteins** with Src-homology-2 domains, in in vitro systems, micro-organisms, eukaryotic cells, whole animals and human beings.

SUMM Phosphorylation of **proteins** is a fundamental mechanism for regulation of many cellular processes. Although **protein** phosphorylation at serine and threonine residues is quantitatively dominating in eukaryotic cells, reversible tyrosine phosphorylation seems to play a pivotal. . .

SUMM The regulation of **protein** tyrosine phosphorylation in vivo is mediated by the opposing actions of **protein** tyrosine kinases (PTKs) and **protein** tyrosine phosphatases (PTPases). The level of **protein** tyrosine phosphorylation of cellular **proteins** is determined by the balanced activities of PTKs and PTPase (Hunter, 1995, supra).

SUMM The **protein** phosphatases are composed of at least two separate and distinct families (Hunter, T., Cell 58: 1013-1016 (1989)) the **protein** serine/threonine phosphatases and the PTPases.

SUMM Low molecular weight phosphotyrosine-**protein** phosphatase (LMW-PTPase) shows very little sequence identity to the intracellular PTPases described above. However, this enzyme belongs to the PTPase. . .

SUMM . . . more than 500 different species will be found in the human genome, i.e. close to the predicted size of the **protein**

tyrosine kinase superfamily (Hanks and Hunter, FASEB J. 9: 576-596 (1995)).

SUMM PTPases are the biological counterparts to **protein** tyrosine kinases (PTKs). Therefore, one important function of PTPases is to control, down-regulate, the activity of PTKs. However, a more. . . dephosphorylation of the C-terminal tyrosine of Fyn and Lck (Chan et al., Annu. Rev. Immunol. 12: 555-592 (1994)). Dual specificity **protein** tyrosine phosphatases (dsPTPases) define a subclass within the PTPases family that can hydrolyze phosphate from phosphortyrosine as well as from. . . His--Cys--Xxx--Xxx--Gly--Xxx--Xxx--Arg (SEQ ID NO: 2). At least three dsPTPases have been shown to dephosphorylate and inactivate extracellular signal-regulated kinase (ERKs)/mitogen-activated **protein** kinase (MAPK): MAPK phosphatse (CL100, 3CH134) (Charles et al., Proc. Natl. Acad. Sci. USA 90: 5292-5296 (1993)); PAC-1 (Ward et. . .

SUMM . . . domains and PTB domains primarily act as docking molecules with little or no catalytic activity. In other words, tyrosine phosphorylated **proteins** have the capacity to bind other **proteins** containing SH2 domains or PTB domains thereby controlling the subcellular location of signalling molecules. There appears to be a significant. . .

SUMM In an early study, vanadate was found to inhibit **protein** -tyrosine phosphatases in mammalian cells with a concomitant increase in the level of phosphotyrosine in cellular **proteins** leading to transformation (Klarlund, Cell 41: 707-717 (1985)). Vanadium-based phosphatase inhibitors are relatively unspecific. Therefore, to assess the importance of. . .

SUMM . . . Mooney and Anderson, J. Biol. Chem. 264: 6850-6857 (1989)), with the tri-phosphorylated tyrosine-1150 domain being the most sensitive target for **protein**-tyrosine phosphatases (PTPases) as compared to the di- and mono- phosphorylated forms (King et al, Biochem. J. 275: 413-418 (1991)). It. . .

SUMM . . . be obtained in adipocytes (Fantus et al., Biochemistry 28: 8864-8871 (1989); Eriksson et at., Diabetologia 39: 235-242 (1995)) and skeletal **muscle** (Leighton et al., Biochem. J. 276: 289292 (1991)). In addition, recent studies show that a new class of peroxovanadium compounds. . .

SUMM . . . signalling in a rat hepatoma cell line (Kulas et al., J. Biol. Chem. 270: 2435-2438 (1995)). A suppression of LAR **protein** levels by about 60 percent was paralleled by an approximately 150 percent increase in insulin-induced auto-phosphorylation. However, only a modest. . .

SUMM . . . the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family **protein** -tyrosine kinase (Mustelin et al., Proc. Natl. Acad. Sci. USA 86: 6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. USA. . . to T-cell activation. In a recent study it was found that recombinant p56.sup.lck specifically associates with recombinant CD45 cytoplasmic domain **protein**, but not to the cytoplasmic domain of the related PTP.alpha. (Ng et al, J. Biol. Chem. 271: 1295-1300 (1996)). The. . . mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosine. In immature B cells, another member of the Src family **protein**-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri et al., J. Biol.. . .

SUMM . . . fibroblasts grow on appropriate substrates, seem to mimic, at least in part, cells and their natural surroundings. Several focal

adhesion **proteins** are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, Neuron 11, 551-564 (1993)). However, aberrant tyrosine phosphorylation of these **proteins** can lead to cellular transformation. The intimate association between PTPases and focal adhesions is supported by the finding of several. . . PTPD1 (M.o slashed.ller et al., Proc. Natl. Acad. Sci. USA 91: 7477-7481 (1994)). The ezrin-like domain show similarity to several **proteins** that are believed to act as links between the cell membrane and the cytoskeleton. PTPD1 was found to be phosphorylated. . .

SUMM PTPases may oppose the action of tyrosine kinases, including those responsible for phosphorylation of focal adhesion **proteins,** and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool et. . .

SUMM . . . et al., J. Biol. Chem. 260: 4653-4660 (1985); Lau et al., J. Biol. Chem. 262:1389-1397 (1987); Lau et al., Adv. **Protein** Phosphatases 4: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when. . .

SUMM In preferred embodiments, the compounds of the invention modulate the activity of **protein** tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s). In one preferred embodiment the compounds of the invention act as inhibitors of PTPases, e.g. **protein** tyrosine phosphatases involved in regulation of tyrosine kinase signalling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signalling pathways via. . .

SUMM . . . The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis, stimulation of the immune system; treatment of retardation, acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein** **catabolic** responses after major surgery; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidio-blastosis; Adjuvant treatment for ovulation induction; stimulation of thymic development and prevention the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling and. . .

SUMM Phosphotyrosine recognition units/tyrosine phosphate recognition units/pTyr recognition units are defined as areas or domains of **proteins** or glycoproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not. . .

SUMM PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing **proteins** or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the. . .

SUMM . . . recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of

proteins or glycoproteins with pTyr recognition units (e.g. PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule with a **protein** or glyco-**protein** with pTyr recognition units either via a direct action on the pTyr recognition site or via an indirect mechanism. Examples. . . leading to initiation of normal or abnormal cellular activity; e) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to **proteins** or glycoproteins with pTyr leading to initiation of normal or abnormal cellular activity.

DETD A mixture of the above acetonitrile (2.50 g, 15 mmol), **ammonium chloride** (1.60 g, 30 mmol) and sodium azide (1.94 g, 30 mmol) in N,N'-dimethylformamide (25 ml) was stirred at 125.degree. C.. . .

DETD A mixture of the above acetonitrile (5.40 g, 32 mmol), **ammonium chloride** (2.59 g, 48 mmol) and sodium azide (3.15 g, 48 mmol) in N,N'-dimethylformamide (100 ml) was stirred at 125.degree. C.. . .

DETD . . . full-length sequence of PTP1B and the intracellular part of PTP.alpha. were introduced into the insect cell expression vector pVL1392. The **proteins** were expressed according to standard procedures. PTP1B was semi-purified by ion exchange chromatography, and PTP.alpha. was purified to apparent homogeneity. . .

L15 ANSWER 32 OF 109 USPATFULL

AB The present invention relates to a method for producing plants with improved agronomic and nutritional traits. Such traits include enhanced nitrogen assimilatory and utilization capacities, faster and more vigorous growth, greater vegetative and reproductive yields, and enriched or altered nitrogen content in vegetative and reproductive parts. More particularly, the invention relates to the engineering of plants modified to have altered expression of key enzymes in the nitrogen assimilation and utilization pathways. In one embodiment of the present invention, the desired altered expression is accomplished by engineering the plant for ectopic overexpression of one of more the native or modified nitrogen assimilatory enzymes. The invention also has a number of other embodiments, all of which are disclosed herein.

AN 1999:113936 USPATFULL
TI Transgenic plants that exhibit enhanced nitrogen assimilation
IN Coruzzi, Gloria M., New York, NY, United States
Brears, Timothy, Durham, NC, United States
PA New York University, New York, NY, United States (U.S. corporation)
PI US 5955651 19990921 <--
AI US 1995-480996 19950607 (8)
RLI Division of Ser. No. US 1994-319176, filed on 6 Oct 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-132334, filed on 6 Oct 1993, now abandoned which is a continuation-in-part of Ser. No. US 1990-514816, filed on 26 Apr 1990, now patented, Pat. No. US 5256558, issued on 26 Oct 1993 which is a continuation-in-part of Ser. No. US 1989-347302, filed on 3 May 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: McElwain, Elizabeth F.
LREP Pennie & Edmonds LLP
CLMN Number of Claims: 11
ECL Exemplary Claim: 6
DRWN 14 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 2499
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5955651 19990921 <--

SUMM . . . asparaginase (ANS; E.C. 3.5.1.1) to produce aspartate and ammonia which then could be utilized in synthesis of amino acids and **proteins** (See FIG. 1).

SUMM . . . Biol. 20:207-218 (transgenic tobacco plants overexpressing soybean GS in tobacco plants). One study has reported observing increases in total soluble **protein** content in transgenic tobacco plants overexpressing the alfalfa GS1 gene. However, since this same study also reported similar increases in total soluble **protein** content in transgenic tobacco plants expressing antisense RNA to the GS1 gene, the relationship between GS1 expression and the increase in soluble **protein** appears unclear (Temple et al., 1993, Mol. Gen. Genet. 236:315-325). One clearly established effect of GS overexpression in plants is. . .

SUMM . . . pattern or level of the nitrogen assimilation or utilization enzyme, altered expression pattern or level of the corresponding mRNA or **protein**, altered nitrogen assimilation or utilization capacities, increased growth rate, enhanced vegetative yield, or improved reproductive yields (e.g., more or larger seeds or fruits). The screening of the engineered plants may involve enzymatic assays and immunoassays to measure enzyme/**protein** levels; Northern analysis, RNase protection, primer extension, reverse transcriptase/PCR, etc. to measure mRNA levels; measuring the amino acid composition, free. . .

SUMM . . . operably linked with sequences encoding a pea glutamine synthetase (GS) gene or a pea asparagine synthetase (AS) gene. RNA and **protein** analyses showed that a majority of the engineered plants exhibited ectopic, overexpression of GS or AS. The GS or AS. . .

DRWD . . . and a sequence encoding a small subunit of a plant or E. coli NADH-GOGAT, containing the NADH-binding domain. The chimeric **protein** encodes a bispecific or bifunctional GOGAT enzyme which can utilize either Fd or NADH as the reductant.

DRWD FIG. 5. Analysis of GS **Protein** in Primary (T1) Transformants Containing GS Transgenes. Top panel: Western analysis of GS polypeptides in primary transformants. Lanes 1 and. . . are shown (as percentages relative to controls =(100%)) below the Western panel. Bottom panel: Coomassie staining of RUBISCO large subunit **protein** demonstrating approximately equal loading of samples. ctGS-chloroplastic GS2 (.about.45 kD); cyGS-cytosolic GS (.about.38 kD).

DRWD FIG. 6. Analysis of GS **Protein**, RNA and Holoenzyme from T2 Progeny Transgenic Plants Containing Pea GS Transgenes. Of the four T2 plants from each primary. . . Panel A (upper): Western analysis of GS polypeptides in transgenic plants. Panel A (lower): Coomassie staining of RUBISCO large subunit **protein** to show approximately equal loading of samples. Panel B (upper): Northern blots hybridized with the approximate cDNA probes for GS1. . .

DRWD FIG. 7A. Activity Gel Analysis of GS Holoenzymes. **Protein** extracts from pea chloroplast (PC), pea root (PR), tobacco chloroplast (TC) and tobacco roots (TR) demonstrating the migration of chloroplastic- and cytosolic-enriched GS **protein** samples relative to the migration of the holoenzymes of GS1 and GS3A overexpressing plants. Lane 1: pea chloroplast **protein** (PC)

has GS holoenzyme B only; lane 2: pea root **protein** (PR) has GS holoenzyme C only; lane 3: tobacco chloroplast **protein** (TC) has GS holoenzyme B only; lane 4: tobacco root **protein** has GS holoenzyme C only. Lane 5: **protein** from plant Z17-7 (carrying the 35S-GS3A construction) has GS holoenzymes A* and B; lane 5: **protein** from plant Z3-1 (carrying the 35S-GS1 construction) has GS holoenzymes B and C.

DRWD FIG. 7B. Western Analysis of GS **Proteins** Isolated from GS Holoenzymes A*, B, and C. Holoenzymes A* and C observed in transgenic tobacco overexpressing GS3A and GS1 were excised from non-denaturing gels, re-extracted in **protein** isolation buffer, and electrophoresed under denaturing conditions for Western analysis using GS antibodies. Lane 1: tobacco leaf **protein** as control; lane 2: GS holoenzyme A* from Z17-7; lane 3: isolated chloroplast GS2 (holoenzyme B) as control; lane 4:. . .

DRWD FIG. 8. Western and Northern Analysis of GS **Protein** and RNA in Transgenic Plants Selected for Growth Analysis Ectopically Expressing either Cytosolic GS1 or GS3A. Upper panel: Western blot for GS **proteins**. Lower panel: Northern blot for GS mRNA. P1 and T1 are pea and tobacco leaf controls. Lanes 1 and 2,. . .

DRWD FIGS. 11A and 11B. Linear relationship between GS activity and plant fresh weight or total leaf **protein**. T2 progenies of primary transformants which showed no segregation of the Kan.sup.R phenotype associated with the transgene were selected for. . . of total leaf

GS

as determined by the transferase assay (B. M. Shapiro, et al., Methods Enzymol. 17A:910 (1970)) and **protein**/gram fresh weight. Plants analyzed were: Control, SR1 untransformed tobacco; Z54-4 co-suppressed by GS2; Z17-7 overexpressing GS3A; Z3-1 overexpressing GS1. FIG. 11A; Plant fresh weight vs. GS activity. FIG. 11B; **protein**/gm fresh weight vs. GS activity.

DETD . . . present invention may involve engineering plants with ectopic overexpression of enzymes catalyzing the use of glutamine, glutamate

and

asparagine in **catabolic** reactions. In a preferred embodiment, a plant is engineered for the ectopic overexpression of asparaginase.

DETD . . . tissues and organs are desired, promoters such as those of the ribulose bisphosphate carboxylase (RUBISCO) genes or chlorophyll a/b binding **protein** (CAB) genes may be used; where expression in seed is desired, promoters such as those of the various seed storage **protein** genes may be used; where expression in nitrogen fixing nodules is desired, promoters such those of the legehemoglobin or nodulin. . .

DETD . . . heat shock genes, the defense responsive gene (e.g., phenylalanine ammonia lyase genes), wound induced genes (e.g., hydroxyproline rich cell wall **protein** genes), chemically-inducible genes (e.g., nitrate reductase genes, gluconase genes, chitinase genes, etc.), dark-inducible genes (e.g., asparagine synthetase gene (Coruzzi and. . .

DETD . . . 3) enzymatic assays for detecting enzyme or ribozyme activity, where such gene products are encoded by the gene construct; 4) **protein** gel electrophoresis, Western blot techniques, immunoprecipitation, or enzyme-linked immunoassays, where the gene construct products are **proteins**. Additional techniques, such as in situ hybridization, enzyme staining, and immunostaining, also may be used to detect the presence or. . .

DETD . . . for suppression of a target gene, transformed plants are examined for those expressing the target gene product (e.g., RNA or **protein**) at reduced levels in various tissues. The plants exhibiting the desired physiological changes, e.g., ectopic GS

overexpression or GS suppression,. . .
DETD . . . herein means any one or any mix of the ammonium salts commonly used as plant nitrogen fertilizer, e.g., ammonium nitrate, **ammonium chloride**, ammonium sulfate, etc.
DETD . . . of the transformed plants may be for improved agronomic characteristics (e.g., faster growth, greater vegetative or reproductive yields, or improved **protein** contents, etc.), as compared to unengineered progenitor plants, when cultivated under nitrogen non-limiting growth conditions (i.e., cultivated using soils or. . .

DETD . . . plants engineered with the alterations in nitrogen assimilation or utilization processes may exhibit improved nitrogen contents, altered amino acid or **protein** compositions, vigorous growth characteristics, increased vegetative yields or better seed yields and qualities. Engineered plants and plant lines possessing such. . . amino acid content of the plant; 8) the free amino acid content of the fruit or seed; 9) the total **protein** content of the plant; and 10) the total **protein** content of the fruit or seed. The procedures and methods for examining these parameters are well known to those skilled. . .
DETD . . . contents than non-suppressed plants. (See Knight and Langston-Unkefer, Science 241:951-954). GS suppressed plants may also have altered amino acid or **protein** contents, making such plants useful in preparation of special dietary foods. Further, all the engineered plants disclosed herein may also. . .
DETD . . . herein show that constitutive overexpression of a heterologous GS subunit for cytosolic GS leads to increases in GS mRNA, GS **protein**, total GS activity, native GS holoenzyme, and, in one case, to the production of a novel GS holoenzyme. Transformed plants. . . significant growth advantage compared to wild type. They grow faster, attain a higher final fresh weight and have more soluble **proteins** than untransformed progenitor plants during the vegetative stage of their development. In some instances, however, overexpression of cytosolic GS and/or. . . GS gene (i.e., co-suppression). Such GS co-suppressed plants may show poorer growth characteristics, but may have altered amino acid and **protein** contents due to shunting of nitrogen into other nitrogen assimilation/metabolism pathways.
DETD 6.1.4. GS **PROTEIN** AND ENZYME ACTIVITY ANALYSIS
DETD Soluble **proteins** were extracted from tobacco and pea leaf tissue as previously described (Tingey and Coruzzi, 1987, Plant Physiol. 84:366-373). **Proteins** were denatured and separated in 12% acrylamide by SDS-PAGE and electroblotted onto nitrocellulose. Western analysis was undertaken using the ProtoBlot. . .
DETD . . . primary transformants (FIG. 6, lanes 9-14). Western blot analysis of these plants confirmed the low abundance of the chloroplast GS2 **protein** (FIG. 6, panel A) and non-denaturing GS activity gel analyses confirmed the reduced abundance of the GS2 holoenzyme (FIG. 6,. . . of a pea GS2 transgene. In addition, the pea GS2 transgene was also silenced. Levels of cytosolic GS mRNA and **protein** were unaffected in these GS2 co-suppressed plants.
DETD . . . of Z17. Transformant Z17-12 is co-suppressed for GS enzyme activity (27% of wild-type) and both chloroplastic GS2 and cytosolic GS

proteins are low (FIG. 5, lane 2) compared to wild-type tobacco (FIG. 5, lane TL). By contrast, transformant Z17-6 has elevated levels of total GS activity (127%) and increased levels of cytosolic GS **protein** (FIG. 5, lane 1) compared to wild-type tobacco (FIG. 5, lane TL). Analysis of the T2 progeny of other independent transformants revealed additional transformants to be down-regulated for cytosolic GS **protein** (Z17-9B and Z17-10; FIG. 6, Panel A, lanes 6 and 7), while others had elevated levels of cytosolic GS (Z17-7. . .
analysis
(FIG. 6, panel A, lanes 3-5) and GS activity assays (Table 1). Non-denaturing GS activity gel analysis of soluble **proteins** from these Z17 transformants which overexpress cytosolic GS3A indicates the existence of a novel GS holoenzyme (band A*, FIG. 6,. . .

DETD . . . of these Z3 transformants are shown in FIG. 6. Both Z3-1 and Z3-2 show an increased abundance of cytosolic GS **protein** (FIG. 6, panel A, lanes 1 and 2) and this is reflected by the increased
levels
of GS mRNA (FIG.. . .

DETD . . . transgenic plants was repeated in non-denaturing activity gels including for comparison, lanes of pea root (PR) and tobacco root (TR) **protein** which are enriched for the cytosolic GS holoenzyme (band C) FIG. 7A, lanes 2 and 4), and extracts derived from. . . composition of the GS activity bands A*, B, and C, these bands were excised from preparative gels, and the extracted **proteins** were reloaded on a denaturing SDS gel followed by Western blot analysis for GS subunits (FIG. 7B). This analysis revealed. . . GS2 subunits. It is possible that GS activity band A* represents the association of transgenic GS3A subunits with a chaperonin-type **protein**, but attempts to dissociate such a complex with ATP were unsuccessful. Consequently, the nature of the novel GS holoenzyme remains. . .

DETD Plant growth analysis was undertaken on the T2 progeny plants analyzed for GS **protein** and RNA in FIG. 8. Individual T2 plants were grown in white sand and growth was assessed by fresh weight. . .

DETD 6.2.11. CORRELATION BETWEEN GS ACTIVITY AND FINAL FRESH WEIGHT AND
TOTAL
PROTEIN

DETD . . . controls by 1.5-times and 2-times, respectively. For these
same
individual T2 plants, a linear relationship also exists between total leaf **protein** (.mu.g **protein**/gm fresh weight) and leaf GS activity. Plants expressing the highest levels of GS activity (284%) had 1.5-fold higher levels of soluble **protein**/gram fresh weight compared to controls (FIG. 11B). An unpaired T-test analysis of this data revealed that the GS overexpressing lines (Z3-1,Z17-7) had significantly greater GS activity, fresh weight, and leaf soluble **protein** with a p value of <0.0001, with the exception of fresh weight for Z17-7 whose p value was 0.0007. Similarly the line co-suppressed by GS2 (Z54-4) had significantly less GS activity, fresh weight, and leaf soluble **protein** than control SR1 with a p value of <0.0001. The GS activity profiles of the GS overexpressing T2 lines used. . .

DETD . . . homologs may be more complex than the overexpression of genes for which there are no homologs, such as viral coat **protein** and BT toxin genes (Powell-Abel et al., 1986, Science 232:738-743;
Vaeck
et al., 1987, Nature 328:33-37). This is due to. . . cytosolic GS which were successfully overexpressed (GS1 and GS3A), the
overexpression
resulted not only in over production of GS RNA, **protein** and enzyme, but also in a phenotype of improved nitrogen use efficiency.

DETD . . . of the pea gene for cytosolic GS1 in tobacco gives a clear phenotype of increased GS activity, increased cytosolic GS **protein**, and high levels of transgene mRNA. Furthermore, the GS1 **protein** assembles into a GS holoenzyme similar in size and charge to native pea cytosolic GS. In transgenic plants overexpressing cytosolic. . . of the overexpressed cytosolic subunits to be released from an assembling chaperonin. Indeed, the close association of GS with groEL-like **proteins** has previously been observed in pea (Tsuprun et al., 1992, Biochim. Biophys. Acta 1099:67-73). However, our attempts to dissociate the. . .

DETD . . . GS activity and an improvement in plant growth and nutritional characteristics. Temple et al. reported increases in GS mRNA and **protein**, but no corresponding increase in GS activity in the transgenic plants (Temple et al., ibid). Hemon et al. reported increased levels of GS mRNA in transgenic plants engineered with GS expression constructs, but found no corresponding increase in GS **protein** or enzyme activity (Hemon et al., ibid). In two other reports, overexpression of GS genes in transgenic plants did result. . .

DETD . . . unstable, the AS enzyme has never been purified to homogeneity and antibodies for plant AS were not available for AS **protein** analysis. In addition, in vitro assay detected no AS activity due to enzyme instability.

DETD . . . for nutrient availability and nitrogen is typically the most critical nutrient at this time due to the synthesis of new **proteins** in expanding and enlarging tissues. Nitrogen assimilated and accumulated at this time is subsequently recycled in the plant and deposited. . .

CLM What is claimed is:

. . . amino acid content in the whole plant, vii) greater free amino acid content in the fruit or seed, viii) greater **protein** content in seed or fruit, or ix) greater **protein** content in a vegetative tissue, than that of a progenitor plant which does not have the gene construct, when the. . .

. . . amino acid content in the whole plant, vii) greater free amino acid content in the fruit or seed, viii) greater **protein** content in seed or fruit, or ix) greater **protein** content in a vegetative tissue, than that of a progenitor plant which does not have the gene construct, when the. . .

. . . amino acid content in the whole plant, vii) greater free amino acid content in the fruit or seed, viii) greater **protein** content in seed or fruit, or ix) greater **protein** content in a vegetative tissue, than a progenitor plant which does not have the gene construct, when the transgenic plant. . .

L15 ANSWER 33 OF 109 USPATFULL

AB Compounds of formula (I) are growth hormone releasing peptide mimetics which are useful for the treatment and prevention of osteoporosis. ##STR1##

AN 1999:92802 USPATFULL

TI Dipeptides which promote release of growth hormone

IN Carpino, Philip A., Groton, CT, United States
Dasilva-Jardine, Paul A., Providence, RI, United States
Lefker, Bruce A., Gales Ferry, CT, United States
Ragan, John A., Gales Ferry, CT, United States

PA Pfizer Inc, New York, NY, United States (U.S. corporation)

PI US 5936089 19990810 <--
WO 9638471 19961205 <--

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AI      US 1997-973268          19971126 (8)
        WO 1995-IB410           19950529
                                19971126  PCT 371 date
                                19971126  PCT 102(e) date
DT      Utility
FS      Granted
EXNAM   Primary Examiner: Ramsuer, Robert W.
LREP    Richardson, Peter C., Ginsburg, Paul H., Speer, Raymond M.
CLMN    Number of Claims: 14
ECL     Exemplary Claim: 1
DRWN    No Drawings
LN.CNT 5362
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI      US 5936089              19990810                              <--
        WO 9638471  19961205                                          <--
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SUMM 1. Increased rate of **protein** synthesis in all cells of the body;

SUMM . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.

SUMM . . . hormone may be summarized as follows: stimulating growth hormone release in elderly humans; treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids, treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating bone fracture repair,. . . syndrome, sleep disorders, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein** **catabolic** response after a major operation; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . adjunctive therapy for patients on chronic hemodialysis; treatment of immunosuppressed patients and to enhance antibody response following vaccination; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodeling, and. . .

SUMM . . . synergistic stimulation of GH release when added together with GHRH, inability to further increase GH secretion by GHRP-6, sensitivity to **protein** kinase C inhibitors, and selective stimulation of biphasic calcium flux in GH-containing cells.

DETD . . . generates the nitro amine of formula 60. Reduction of the nitro functionality to the corresponding amine using iron powder and **ammonium chloride** in refluxing aqueous ethanol is one of many suitable literature procedures (see March, J., p. 1103-4, Advanced Org. Chem.; Reactions,. . .

DETD . . . and 1.5 mL of water was added 110 mg (1.96 mmol) of iron powder, and 12 mg (0.22 mmol) of **ammonium chloride**. The mixture was refluxed for 45 min, and another 110 mg of iron powder, and 24 mg of **ammonium chloride** was added to the reaction mixture and refluxing continued for another 1 h. The hot solution was filtered through celite. . .

DETD . . . room temperature and was stirred for 17 h. The reaction mixture

was quenched by adding 150 mL of saturated aqueous **ammonium chloride**. The organic layer was separated and the aqueous phase was extracted with ether. The combined organic extracts were washed once. . .

DETD To a mixture of 255 mg (0.44 mmol) of 52E, 118 mg (2.21 mmol) of **ammonium chloride** and 400 mg (3.09 mmol) of diisopropylethylamine in 4 mL of methylene chloride was added 235 mg (0.53 mmol) of. . .

L15 ANSWER 34 OF 109 USPATFULL

AB This invention is directed to the pharmaceutical use of phenyl compounds, which are linked to an aryl moiety by various linkages, for inhibiting tumor necrosis factor. The invention is also directed to the compounds, their preparation and pharmaceutical compositions containing these compounds. Furthermore, this invention is directed to the pharmaceutical use of the compounds for inhibiting cyclic AMP phosphodiesterase.

AN 1999:92693 USPATFULL

TI Compounds containing phenyl linked to aryl or heteroaryl by an aliphatic- or heteroatom-containing linking group

IN Fenton, Garry, Dagenham, United Kingdom
Morley, Andrew David, Dagenham, United Kingdom
Palfreyman, Malcolm Norman, Dagenham, United Kingdom
Ratcliffe, Andrew James, Dagenham, United Kingdom
Sharp, Brian William, Dagenham, United Kingdom
Thurairatnam, Sukanthini, Dagenham, United Kingdom
Vacher, Bernard Yvon Jack, Dagenham, United Kingdom
Ashton, Michael John, Dagenham, United Kingdom
Cook, David Charles, Dagenham, United Kingdom
Hills, Susan Jacqueline, Dagenham, United Kingdom
McFarlane, Ian Michael, Dagenham, United Kingdom
Vicker, Nigel, Dagenham, United Kingdom

PA Rhone-Poulenc Rorer Limited, West Malling, United Kingdom (non-U.S. corporation)

PI US 5935978 19990810 <--

AI US 1993-98178 19930728 (8)

RLI Continuation-in-part of Ser. No. WO 1992-GB153, filed on 28 Jan 1992, now abandoned

PRAI GB 1991-1777 19910128
GB 1991-17727 19910816
GB 1992-15989 19920728
GB 1992-16005 19920728
GB 1992-16006 19920728
GB 1992-16008 19920728
GB 1992-16764 19920807
GB 1993-10633 19930521
GB 1993-10938 19930527
GB 1993-11281 19930601
GB 1993-14847 19930716

DT Utility

FS Granted

EXNAM Primary Examiner: Davis, Zinna Northington

LREP Parker, III, Raymond S., Savitzky, Martin F.

CLMN Number of Claims: 36

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 4870

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5935978 19990810 <--

SUMM . . . compounds, their preparation, pharmaceutical compositions

containing these compounds, and their pharmaceutical use in the treatment of disease states associated with **proteins** that mediate cellular activity.

SUMM The principal in vivo actions of TNF can be broadly classified as inflammatory and **catabolic**. It has been implicated as a mediator of endotoxic shock, inflammation of joints and of the airways, immune deficiency states,. . .

SUMM . . . the anti-coagulant activity of vascular endothelial cells. The cachexia associated with certain disease states is mediated through indirect effects on **protein** catabolism. TNF also promotes bone resorption and acute phase **protein** synthesis.

DETD . . . hour, allowed to warm to room temperature and left to stand overnight. The mixture is then quenched with 10% aqueous **ammonium chloride** solution (150 mL), the layers separated and the aqueous layer further extracted with ethyl acetate (2.times.100 mL). The combined organic. . .

DETD . . . and then it is stirred for a further 6 hours. It is then treated with a saturated aqueous solution of **ammonium chloride** (300 mL), and concentrated in vacuo to low volume. The aqueous residue is extracted with ethyl acetate (2.times.200 mL). The. . .

DETD . . . for a further 2 hours in the cold, the mixture is filtered, and the filtrate is washed with saturated aqueous **ammonium chloride** solution. The organic phase is dried over sodium sulfate and evaporated. The resulting residue is subjected to flash chromatography, eluting. . .

DETD . . . to room temperature and the solution is stirred for a further 2 hours. The reaction mixture is treated with aqueous **ammonium chloride** solution (50 mL) and the solution is extracted with diethyl ether (2.times.200 mL). The combined extracts are dried and concentrated,. . .

DETD . . . 15 minutes, and stirred for a further 1 hour 30 minutes at -75.degree. C. The solution is treated with aqueous **ammonium chloride** solution and extracted with ethyl acetate (3.times.100 mL). The organic layers are combined, washed with brine, dried and concentrated to. . .

DETD . . . 30 minutes. The resulting mixture is then allowed to warm to room temperature overnight, and then treated with saturated aqueous **ammonium chloride** solution (200 mL). The layers are separated and the aqueous layer is further extracted with ethyl acetate (3.times.300 mL). The. . .

DETD . . . is trimmed off and the endothelial layer on the intimal surface is removed by rubbing with a cotton swab. Smooth **muscle** strips are plucked from the aorta and 25 g are homogenized using a Waring Blender in homogenization buffer (20 mM. . .

DETD 3. Effects of compounds on tracheal smooth **muscle** contractility.

L15 ANSWER 35 OF 109 USPATFULL

AB Cardiac output is measured utilizing a catheter in conjunction with the indicator dilution technique. Non-thermal analyte-containing fluid is used as the injectate. This fluid is biocompatible with and metabolizable within the body of the patient. An analyte concentration sensor is mounted upon the catheter and located downstream within the bloodstream from the port from which the analyte-containing fluid is expressed. Because of the matching of rapid concentration sensor response with an analyte-containing fluid which is metabolizable, the

measurement of cardiac output may be carried out as often as about one to three minutes in conjunction with an infusion interval substantially less than the measurement frequency interval. The analyte-containing fluids are selected from a group consisting of ammoniacal fluid, heparin, ethanol, a carbon dioxide releasing fluid, glucose, and anesthesia agent. The system performs in conjunction with a microprocessor-driven controller which automates the measurement procedure and provides a display of cardiac output and various cardiovascular parameters.

AN 1999:84507 USPATFULL
TI Cardiac output measurement with metabolizable analyte containing fluid
IN Eggers, Philip E., Dublin, OH, United States
Huntley, Scott P., Danville, CA, United States
Khalil, Gamal Eddin, Redmond, WA, United States
PA Cardiox Corporation, Menlo Park, CA, United States (U.S. corporation)
PI US 5928155 19990727 <--
AI US 1998-40167 19980317 (9)
RLI Continuation-in-part of Ser. No. US 1997-792967, filed on 24 Jan 1997, now patented, Pat. No. US 5788647
DT Utility
FS Granted
EXNAM Primary Examiner: O'Connor, Cary; Assistant Examiner: Winakur, Eric F.
LREP Mueller and Smith, LPA
CLMN Number of Claims: 62
ECL Exemplary Claim: 1
DRWN 39 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 2843
PI US 5928155 19990727 <--
DETD . . . choice with current thermodilution techniques. This preferred embodiment also employs the noted ammoniacal fluid as the analyte-containing fluid, for example, **ammonium chloride**. The indicator or analyte concentration of the analyte-containing fluid for this selection will be the combined content of ammonia gas. . .
DETD . . . pp 449-460, 1979). Under resting conditions, most blood ammonia/ammonium is of dietary origin. Normal digestive processes generate ammonia/ammonium from ingested **protein**, while bacteria in the gastrointestinal tract generate ammonia/ammonium by metabolizing **protein** products of dietary **protein** digestion and urea. An illustration of the major organs of ammonia/ammonium formation, utilization and circulation is presented in FIG. 4. . . or gastrointestinal tract as represented at arrow 102 and block 104. Ammonia generated in the gut as at 74 from **protein** digestion and deamination of glutamine (GLN) enters the portal venous circulation as represented at arrows 106 and 108 and is. . . by arrows 112-114. Metabolic interaction with the kidney as at block 116 is represented at arrows 118 and 119, while **catabolic** ammonium is excreted as represented at arrow 120 and block 122. Transport to and from the brain with respect to the blood pool is represented at block 124 and arrows 126-128. A similar metabolic interrelationship with respect to skeletal **muscle** is represented at block 130 and arrows 132 and 133. Exercise induced hyperammonemia will witness a transfer of ammonium ion. . . of Sports Medicine, 649 Vol. 11, pp 5129-5142 (1990). Under conditions typical of patients in an intensive care unit, resting **muscles** take up ammonia/ammonium from the circulating blood wherein the substance enters into **protein** synthesis via ketoglutaric and glutamic acid. When the **muscle**

begins working again, ammonia/ammonium is once again released from the **muscle** into the bloodstream. If additional ammonia/ammonium (in the form of an ammonium salt solution) is injected into a peripheral vein,. . . brought directly to the tissue via the blood where it may be retained and eventually used for amino acid and **protein** synthesis. See: Furst, P., et al., "Nitrogen Balance After Intravenous and Oral Administration of Ammonia Salts in Man," Journal of. . .

DETD . . . liquid 578. Where the analyte-containing fluid is an ammoniacal fluid, the liquid 578 may be a solution containing 0.1 molor **ammonium chloride**. That liquid 578 reaches equilibrium with a blood carried ammonium ion flow across the membrane 568 to change or alter. . .

L15 ANSWER 36 OF 109 USPATFULL

AB Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

AN 1999:18720 USPATFULL

TI Altered polypeptides with increased half-life

IN Presta, Leonard G., San Francsico, CA, United States
Snedecor, Bradley R., Portola Valley, CA, United States

PA Genentech, Inc., So. San Francisco, CA, United States (U.S. corporation)

PI US 5869046 19990209 <--

AI US 1995-422092 19950414 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Eisenschenk, Frank C.; Assistant Examiner: Rabin, Evelyn

LREP Dreger, Walter H.

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN 4 Drawing Figure(s); 3 Drawing Page(s)

LN.CNT 3287

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5869046 19990209 <--

SUMM . . . (pFc') fragment of human IgG also produced by trypsin digestion of the Fc fragment was rapidly eliminated, indicating that the **catabolic** site of IgG is located in the CH2 domain. Ellerson et al., J. Immunol., 116: 510 (1976); Yasmeen et al.,. . .

SUMM The **catabolic** rates of IgG variants that do not bind the high-affinity Fc receptor FcRI or C1q are indistinguishable from the rate of clearance of the parent wild-type antibody, indicating that the **catabolic** site is distinct from the sites involved in FcRI or C1 q binding. Wawrzynczak et al., Molec. Immunol., 29: 221. . .

SUMM Staphylococcal **protein** A-IgG complexes were found to clear more rapidly from the serum than uncomplexed IgG molecules. Dima et al., Eur. J.. . . on the pharmacokinetics of the Fc-hinge fragment. The authors showed that the site of the IgG1 molecule that controls the **catabolic** rate (the "**catabolic** site") is located at the CH2-CH3 domain interface and overlaps with the Staphylococcal **protein** A binding site. See also WO 93/22332 published Nov. 11, 1993. The concentration catabolism phenomenon is also studied in Zuckier. . .

SUMM WO 94/04689 discloses a **protein** with a cytotoxic domain, a ligand-binding domain and a peptide linking these two domains comprising an IgG constant region domain having the property of increasing the half-life of the **protein** in mammalian serum.

SUMM A stereo drawing of a human Fc fragment and its complex with fragment B of **Protein** A from Staphylococcus aureus is provided by Deisenhofer, Biochemistry, 20: 2364 (1981).

DETD . . . as is well known to those skilled in the art of antibody technology. Examples of such polypeptides are peptides and **proteins**, whether from eukaryotic sources such as, e.g., yeast, avians, plants, insects, or mammals, or from bacterial sources such as, e.g.,. . .

DETD . . . hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as **Protein** C; atrial naturietic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA);. . . a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial **protein**, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; **protein** A or D; rheumatoid factors; a neurotrophic factor such as brain-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3,. . . TGF-.beta.2, TGF-.beta.3, TGF-.beta.4, or TGF-.beta.5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding **proteins**; CD **proteins** such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic **protein** (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs),. . . IL-1 to IL-10; an anti-HER-2 antibody without a native Fc region of an IgG; superoxide dismutase; T-cell receptors; surface membrane **proteins**; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport **proteins**; homing receptors; addressins; regulatory **proteins**; antibodies without a native Fc region of an IgG; and fragments of any of the above-listed polypeptides.

DETD Libraries are screened with probes designed to identify the gene of interest or the **protein** encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to. . .

DETD . . . In some preferred embodiments, the nucleic acid sequence includes the polypeptide of interest's signal sequence. Nucleic acid having all the **protein** coding sequence is obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for. . .

DETD . . . insertions include insertions to the internal portion of the polypeptide of interest, as well as N- or C-terminal fusions with **proteins** or peptides containing the desired epitope that will result, upon fusion, in an increased half-life.

DETD . . . bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for **protein** production, generally an expression vector of the type typically employed for transformation of an appropriate host.

DETD Expression and cloning vectors should contain a selection gene, also

termed a selectable marker. This gene encodes a **protein** necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode **proteins** that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or. . .

DETD . . . drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a **protein** conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et. . .

DETD . . . that encodes the polypeptide variant. Amplification is the process by which genes in greater demand for the production of a **protein** critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the. . .

DETD . . . cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the polypeptide variant, wild-type DHFR **protein**, and another selectable marker such as aminoglycoside 3-phosphotransferase (APH) can be selected by cell growth in medium containing a selection. . .

DETD . . . amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding **proteins** endogenous to the host, with examples of such hosts including E. coli W3110 strain 1A2, which has the complete genotype. . .

DETD . . . Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-**protein** duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound. . .

DETD . . . step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration; optionally, the **protein** may be concentrated with a commercially available **protein** concentration filter, followed by separating the polypeptide variant from other impurities by one or more steps selected from immunoaffinity chromatography,. . . groups), chromatography on Blue-Sepharose, CM Blue-Sepharose, MONO-Q, MONO-S, lentil lectin-Sepharose, WGA-Sepharose, Con A-Sepharose, Ether Toyopearl, Butyl Toyopearl, Phenyl Toyopearl, or **protein** A Sepharose, SDS-PAGE chromatography, silica chromatography, chromatofocusing, reverse phase HPLC (e.g., silica gel with appended aliphatic groups), gel filtration using,. . .

DETD . . . another embodiment, supernatants from systems which secrete recombinant polypeptide variant into culture medium are first concentrated using a commercially available **protein** concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate may be applied to a suitable purification matrix. For example, a suitable affinity matrix may comprise a ligand for the **protein** , a lectin or antibody molecule bound to a suitable support. Alternatively, an anion-exchange resin may be employed, for example, a. . . matrix or substrate having pendant DEAE groups. Suitable matrices include acrylamide, agarose, dextran, cellulose, or other types commonly employed in **protein** purification. Alternatively, a cation-exchange step may be employed. Suitable cation exchangers include

various insoluble matrices comprising sulfopropyl or carboxymethyl groups.. . .

DETD Mammalian polypeptide variant synthesized in recombinant culture is characterized by the presence of non-human cell components, including **proteins**, in amounts and of a character which depend on the purification steps taken to recover the polypeptide variant from culture.. . .

DETD . . . O-acetyl tyrosyl species and 3-nitro derivatives, respectively.
Tyrosyl residues are iodinated using .sup.125 I or .sup.131 I to prepare
labeled **proteins** for use in radioimmunoassay, the chloramine T method described above being suitable.

DETD . . . of seryl or threonyl residues, methylation of the .alpha.-amino
groups of lysine, arginine, and histidine side chains (T. E. Creighton, **Proteins**: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and. . .

DETD . . . the compound tunicamycin as described by Duskin et al., J. Biol. Chem., 257: 3105 (1982). Tunicamycin blocks the formation of **protein**-N-glycoside linkages.

DETD . . . be fused to a second polypeptide and the antibody or fusion thereof may be used to isolate and purify the **protein** to which it binds from a source such as a CD11 or CD18 antigen. In another embodiment, the invention provides. . .

DETD . . . as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; **proteins**, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine. . .

DETD . . . (ip) injections of the relevant antigen and an adjuvant. It may
be useful to conjugate the relevant antigen to a **protein** that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin. . .

DETD . . . the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different **protein** and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as **protein** fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

DETD . . . as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the **protein** used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable. . .

DETD . . . are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as,
for example, **protein** A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DETD . . . E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin **protein**, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of. . .

DETD Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic **protein** chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a. . .

DETD . . . using leucine zippers. Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun **proteins** were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the. . .

DETD . . . tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or variant antibody, and **proteins** that bind to the analyte are denominated binding partners, whether they be antibodies, cell-surface receptors, or antigens.

DETD . . . flanking the F1 origin were removed from pB0475 and DNA coding for anti-CD18 Fab H52, version OZ (Eigenbrot et al., **Proteins**, 18: 49-62 [1994]) was substituted for DNA coding for human growth hormone using the EcoRV and SphI sites. Hence, pH52. . .

DETD . . . g yeast extract certified (Difco.TM. 0127-01-7), 0.19 g MgSO.sub.4 anhydrous or 0.394 g MgSO.sub.4. 7H.sub.2 O (Sigma.TM. M2773), 1.07 g **ammonium chloride** (Sigma.TM. A9434), 0.075 g KCl (Sigma.TM. P5405), 4.09 g NaCl (Sigma.TM. S3014), 120.0 mL of 1M triethanolamine pH 7.4, qs. . .

DETD The supernatant was then passed over a **Protein** G-Sepharose.TM. Fast Flow (Pharmacia) column [0.5 mL bed volume] previously equilibrated by passing 10 mL TE buffer through the column.. . . added to concentrated eluant, and the resulting mixture was re-concentrated to 0.5 mL. SDS-PAGE gels were run to ascertain that **protein** had been produced.

DETD . . . on a reverse- phase PLRP-S.TM. 4.6.times.50 mm column, 8-mm particle size (Polymer Laboratories, Shropshire, UK), maintained at 50.degree. C. The **proteins** were eluted using an increasing linear gradient from 31% B to 41% B. Buffer A contained 0.1% trifluoroacetic acid in. . . and Buffer B contained 0.1% trifluoroacetic acid in HPLC-grade acetonitrile. The flow rate was maintained at 2 mL/min, and the **protein** profile was monitored at 214 nm.

DETD . . . carried out on a Bakerbond carboxy-sulfon (CSX).TM. 50.times.4.6 mm column (J. T. Baker Phillipsburg, N.J.), maintained at 55.degree. C. The **proteins** were eluted using an increasing linear gradient from pH 6.0 to pH 8.0 at a flow rate of 2 mL/min. . .

DETD SDS-PAGE analysis was carried out on precast NoveX.TM. gels (Novex, San Diego, Calif.). The **proteins** were stained using the Morrissey silver stain method. Morrissey, Anal. Biochem., 117: 307-310 (1981).

DETD . . . test (Associates of Cape Cod Inc., Woods Hole, Mass.). Samples containing less than 2 endotoxin units (Eu) per mg of **protein** were used in the pharmacokinetic studies.

DETD . . . 3.5. To this solution, pepsin (1 mg/mL) dissolved in 100 mM sodium citrate buffer, pH 3.5, was added at a pepsin-to-**protein** ratio of 1:12. After 4 hours at room temperature, the mixture's pH was raised to pH 6.4 with 10% NaOH.

DETD . . . described above for the Fab antibody fragment variants. After endotoxin determinations, samples containing less than 2 Eu per mg of **protein** were used in the pharmacokinetic studies set forth below.

L15 ANSWER 37 OF 109 USPATFULL

AB In this disclosure, there are provided materials which completely

degrade in the environment far more rapidly than pure synthetic plastics but which possesses the desirable properties of a thermoplastic: strength, impact resistance, stability to aqueous acid or base, and deformation at higher temperatures. There is provided a method for using the degradable plastic materials in preparing strong, moldable solids. There is further provided a method of making and applications for macromolecular, surface active agents that change the wetting behavior of lignin-containing materials. These surface active agents are used to provide a method of making and applications for synthetic polymers coupled to pieces of a vascular plant using macromolecular surface active agents.

AN   1998:159999 USPATFULL

TI   Biodegradable plastics and composites from wood

IN   Meister, John J., 31675 Westlady Rd., Beverly Hills, MI, United States 48025-3744
        Chen, Meng-Jiu, 901 St. Louis, Apt. #25, Ferndale, MI, United States 48220

PI   US 5852069   19981222   <--

AI   US 1997-942868   19971002 (8)

RLI   Division of Ser. No. US 1995-400891, filed on 8 Mar 1995, now patented, Pat. No. US 5741875 which is a continuation-in-part of Ser. No. US 1993-80006, filed on 21 Jun 1993, now patented, Pat. No. US 5424382 which is a continuation-in-part of Ser. No. US 1991-789360, filed on 8 Nov 1991, now abandoned

DT   Utility

FS   Granted

EXNAM   Primary Examiner: Truong, Duc

LREP   Barnes,Kisselle,Raisch,Choate,Whittemore & Hulbert, P.C.

CLMN   Number of Claims: 3

ECL   Exemplary Claim: 1

DRWN   8 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 2189

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI   US 5852069   19981222   <--

DETD   . . . cellulose, hemicellulose, and lignin, possibly contaminated with the inert "mineral" portion of the plant: starch, lipid, silica bodies, silica stegmata, **protein** bodies, and mucilage.

DETD   TABLE 4

Some Halides Useful in Polymerization of Lignin-Containing Materials.

Calcium Chloride	Magnesium Chloride	Sodium Chloride
Potassium Chloride	Uthium Chloride	**Ammonium Chloride**
Calcium Bromide	Magnesium Bromide	Sodium Bromide
Potassium Bromide	Lithium Bromide	Ammonium Bromide
Calcium Fluoride	Magnesium Fluoride	Sodium Fluoride
Potassium Fluoride		

Lithium Fluoride
Ammonium Fluoride

DETD . . . with brown rot fungus Gloeophyllum trabeum. Three of these fungi are white-rot species that attack and degrade woody materials by **catabolic** activity while the fourth fungus is a brown-rot that acts as a negative control since it attacks woody materials by. . .

L15 ANSWER 38 OF 109 USPATFULL

AB This invention is directed to the pharmaceutical use of phenyl compounds, which are linked to an aryl moiety by various linkages, for inhibiting tumor necrosis factor. The invention is also directed to the compounds, their preparation and pharmaceutical compositions containing these compounds. Furthermore, this invention is directed to the pharmaceutical use of the compounds for inhibiting cyclic AMP phosphodiesterase.

AN 1998:147439 USPATFULL

TI Compounds containing phenyl linked to aryl or heteroaryl by an aliphatic- or

IN Fenton, Garry, Dagenham, United Kingdom
Morley, Andrew David, Dagenham, United Kingdom
Palfreyman, Malcolm Norman, Dagenham, United Kingdom
Ratcliffe, Andrew James, Dagenham, United Kingdom
Sharp, Brian William, Dagenham, United Kingdom
Stuttle, Keith Alfred James, Dagenham, United Kingdom
Thurairatnam, Sukanthini, Dagenham, United Kingdom
Vacher, Bernard Yvon Jack, Dagenham, United Kingdom

PA Rhone-Poulenc Rorer Limited, West Malling, United Kingdom (non-U.S. corporation)

PI US 5840724 19981124 <--

AI US 1997-881888 19970624 (8)

RLI Division of Ser. No. US 1995-484805, filed on 7 Jun 1995, now patented, Pat. No. US 5679696, issued on 21 Oct 1997 which is a division of Ser. No. US 1993-98178, filed on 28 Jul 1993

PRAI GB 1993-11281 19930601

DT Utility

FS Granted

EXNAM Primary Examiner: Davis, Zinna Northington

LREP Parker, III, Raymond S.

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 4810

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5840724 19981124 <--

SUMM . . . compounds, their preparation, pharmaceutical compositions containing these compounds, and their pharmaceutical use in the treatment of disease states associated with **proteins** that mediate cellular activity.

SUMM The principal in vivo actions of TNF can be broadly classified as inflammatory and **catabolic**. It has been implicated as a mediator of endotoxic shock, inflammation of joints and of the airways, immune deficiency states,. . .

SUMM . . . the anti-coagulant activity of vascular endothelial cells. The cachexia associated with certain disease states is mediated through indirect effects on **protein** catabolism. TNF also promotes bone resorption and acute phase **protein** synthesis.

DETD . . . hour, allowed to warm to room temperature and left to stand overnight. The mixture is then quenched with 10% aqueous **ammonium chloride** solution (150 mL), the layers

separated and the aqueous layer further extracted with ethyl acetate (2.times.100 mL). The combined organic. . .

DETD . . . and then it is stirred for a further 6 hours. It is then treated with a saturated aqueous solution of **ammonium chloride** (300 mL), and concentrated in vacuo to low volume. The aqueous residue is extracted with ethyl acetate (2.times.200 mL). The. . .

DETD . . . for a further 2 hours in the cold, the mixture is filtered, and the filtrate is washed with saturated aqueous **ammonium chloride** solution. The organic phase is dried over sodium sulfate and evaporated. The resulting residue is subjected to flash chromatography, eluting. . .

DETD . . . to room temperature and the solution is stirred for a further 2 hours. The reaction mixture is treated with aqueous **ammonium chloride** solution (50 mL) and the solution is extracted with diethyl ether (2.times.200 mL). The combined extracts are dried and concentrated,. . .

DETD . . . 15 minutes, and stirred for a further 1 hour 30 minutes at -75.degree. C. The solution is treated with aqueous **ammonium chloride** solution and extracted with ethyl acetate (3.times.100 mL). The organic layers are combined, washed with brine, dried and concentrated to. . .

DETD . . . 30 minutes. The resulting mixture is then allowed to warm to room temperature overnight, and then treated with saturated aqueous **ammonium chloride** solution (200 mL). The layers are separated and the aqueous layer is further extracted with ethyl acetate (3.times.300 mL). The. . .

DETD . . . is trimmed off and the endothelial layer on the intimal surface is removed by rubbing with a cotton swab. Smooth **muscle** strips are plucked from the aorta and 25 g are homogenized using a Waring Blender in homogenization buffer (20 mM. . .

DETD 3. Effects of compounds on tracheal smooth **muscle** contractility.

L15 ANSWER 39 OF 109 USPATFULL

AB There are disclosed certain novel compounds identified as heterocyclic-fused lactams which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to increase the stature of those afflicted with a lack of a normal secretion of natural growth hormone. Growth promoting compositions containing such heterocyclic-fused lactams as the active ingredient thereof are also disclosed.

AN 1998:92199 USPATFULL

TI Heterocyclic-fused lactams promote release of growth hormone

IN Fisher, Michael H., Ringoes, NJ, United States
Mrozik, Helmut, Matawan, NJ, United States
Schoen, William R., Edison, NJ, United States
Shih, Thomas L., Edison, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5789587 19980804 <--

AI US 1996-744296 19961106 (8)

RLI Division of Ser. No. US 1993-166440, filed on 14 Dec 1993, now patented, Pat. No. US 5606054

DT   Utility
FS   Granted
EXNAM   Primary Examiner: Bond, Robert T.
LREP   Thies, J. Eric, Rose, David L.
CLMN   Number of Claims: 6
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 1767
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5789587   19980804   <--
SUMM   1. Increased rate of **protein** synthesis in all cells of the body;
SUMM   . . . still further use of the disclosed novel heterocyclic-fused lactam growth hormone secretagogues is in combination with IGF-1 to reverse the **catabolic** effects of nitrogen wasting as described by Kupfer, et al, J. Clin. Invest., 91, 391 (1993).
SUMM   . . . These varied uses of growth hormone may be summarized as follows: stimulating growth hormone release in elderly humans; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system; treatment of retardation; acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS. Treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .
DETD   . . . at room temperature overnight, then diluted with 1 L of methylene chloride and washed with 500 mL of saturated aqueous **ammonium chloride**, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over magnesium. . .
DETD   . . . hydride/oil dispersion. After 2 min, 60 mg of N-triphenylmethyl-5-[2-(4'-bromomethylbiphen-4-yl)]tetrazole was added. After an additional 5 min, ice and saturated aqueous **ammonium chloride** solution was added to stop the reaction. The products were extracted with ethyl acetate and purified by PTLC on silica. . .

L15 ANSWER 40 OF 109 USPATFULL
AB   The ATP-ubiquitin-dependent process has been shown to be responsible for the excessive **protein** degradation which occurs in conditions or disease states in which there is severe loss of body mass and negative nitrogen balance has been identified and key constituents in the process identified. A method of inhibiting the accelerated or enhanced proteolysis, a method of identifying inhibitors of the process, multipain and the proteasome inhibitor are the subject of the claimed invention.
AN   1998:88814 USPATFULL
TI   ATP-dependent protease and use of inhibitors for same in the treatment of cachexia and **muscle** wasting
IN   Goldberg, Alfred L., Brookline, MA, United States

PA The President and Fellows of Harvard College, Cambridge, MA, United States (U.S. corporation)
PI US 5786329 19980728 <--
AI US 1996-730310 19961011 (8)
RLI Division of Ser. No. US 1994-262497, filed on 20 Jun 1994, now patented,
Pat. No. US 5565351 which is a division of Ser. No. US 1991-699184, filed on 13 May 1991, now patented, Pat. No. US 5340736
DT Utility
FS Granted
EXNAM Primary Examiner: Patterson, Jr., Charles L.
LREP Sterne, Kessler, Goldstein & Fox P.L.L.C.
CLMN Number of Claims: 3
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 2887
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI ATP-dependent protease and use of inhibitors for same in the treatment of cachexia and **muscle** wasting
PI US 5786329 19980728 <--
AB The ATP-ubiquitin-dependent process has been shown to be responsible for
the excessive **protein** degradation which occurs in conditions or disease states in which there is severe loss of body mass and negative nitrogen. . . .
SUMM Mammalian cells contain at least four proteolytic systems which appear to serve distinct functions in the turnover of cell **proteins**.
In the cytosol, there is a soluble proteolytic pathway that requires ATP
and involves the polypeptide ubiquitin. This multicomponent system catalyzes the selective degradation of highly abnormal **proteins** and short-lived regulatory **proteins**. However, this process also appears to be responsible for the breakdown of most **proteins** in maturing reticulocytes. Boches, F. and A. L. Goldberg, Science, 215:978-980 (1982); Spenser, S. and J. Etlinger, J. Biol. Chem.,. . . et al., J. Biol. Chem., 260:3344-3349 (1985) In cells deprived of insulin or serum, the breakdown of the average cell **proteins** increases up to 2-fold. This accelerated proteolysis involves the lysosomes, which are also the sites for the breakdown of endocytosed and membrane **protein**. Another system by which skeletal **muscle** can increase overall proteolysis involves the Ca.sup.2+ -dependent proteases (calpains I and II). In dystrophic or damaged **muscle** or in normal **muscle** after treatments that raise intracellular Ca.sup.2+, overall **protein** breakdown rises, due mainly to activation of the calpains. In addition, there is a
nonlysosomal degradative system that functions independently of ATP; in erythrocytes, this system catalyzes the selective breakdown of oxidant-damaged **proteins**. The relative importance of these systems in the degradation of different cell components under various conditions in **muscle** is unknown.
SUMM In the process requiring Ub, the first step in degradation of many **proteins** involves their conjugation to this small polypeptide by an ATP-requiring process. The ubiquitinated **proteins** are then degraded by a 1000-1500 kDa (26S) ATP-dependent proteolytic complex, the
Ub-Conjugate-Degrading Enzyme ("UCDEN"). This pathway has been best characterized in reticulocytes, but has also been demonstrated in skeletal **muscle** and other cells. It is believed to be responsible for the rapid degradation of highly abnormal

proteins and many short-lived enzymes or regulatory **proteins**.

SUMM . . . contains 12-15 distinct subunits and three distinct peptidases of different specificities. By itself, the proteasome is unable to degrade ubiquitinated **proteins** and provides most of the proteolytic activity of UCDEN.

SUMM The present invention relates to a method of inhibiting (reducing or preventing) the accelerated breakdown of **muscle proteins** which accompanies various physiological and pathological states and is responsible to a large extent for the loss of **muscle** mass (atrophy) which follows nerve injury, fasting, fever, acidosis and certain endocrinopathies. As described herein, it has been shown that the nonlysosomal ATP-ubiquitin-dependent proteolytic process increases in **muscle** in these conditions and is responsible for most of the accelerated proteolysis which occurs in atrophying **muscles**. This is supported by the demonstration, also described herein, that there is a specific increase in ubiquitin mRNA, induction of mRNA for proteasome and increased ubiquitinated **protein** content in atrophying **muscles** which is not seen in non-**muscle** tissue under the same conditions.

SUMM The present invention further relates to a novel ATP-dependent protease which is involved in degradation of ubiquitinated **proteins**, forms a complex with the proteasome and appears to be part of the 1300-1500 kDa ATP-dependent proteolytic complex (UCDEN referred to as the 1500 kDa complex) which rapidly degrades **proteins** conjugated to ubiquitin. This novel protease, referred to as multipain, appears to play a critical role in the ATP-ubiquitin-dependent pathway.

SUMM Multipain is a multimeric enzyme of molecular weight approximately 500 kDa, which requires ATP hydrolysis for activation and degrades ubiquitinated **proteins** preferentially. This new ATP-dependent enzyme appears to be a thiol protease and has been shown to cleave Ub-conjugated **proteins** to acid-soluble products. Multipain has been identified in **muscle** and shown to play an essential role in the cytosolic pathway which is activated in various forms of **muscle** wasting.

SUMM The present invention further relates to purified multipain, obtained from sources in which it normally is found, such as skeletal **muscle** cells; DNA or RNA encoding multipain; multipain produced by recombinant DNA methods; antibodies specific for the enzyme; methods of using multipain; and multipain inhibitors and their use, particularly for reducing the loss of **muscle** mass which occurs in a variety of diseases or conditions.

SUMM . . . an inhibitor of another component of the 1500 kDa complex can be administered to an individual in whom loss of **muscle** mass occurs (e.g., following nerve injury, fasting, infection or certain endocrinopathies). **Muscle** mass losses in such conditions are due in turn to accelerated breakdown of **muscle proteins**, which has been shown, as described herein, to be due largely to activation of the non-lysosomal ATP-ubiquitin-dependent pathway, in which. . . a multipain inhibitor or an inhibitor of another component of the ATP-dependent proteolytic complex will interfere with or reduce enhanced **protein** breakdown which normally occurs in such conditions. As a result, proteolysis is reduced and **muscle protein** loss occurs to a lesser extent than normally occurs in such conditions. This method of inhibiting multipain or another component of the 1500 kDa complex and, as a result,

of inhibiting destruction of **muscle protein**, can be used in a wide variety of conditions, such as cancer, chronic infectious diseases, fever and **muscle** disuse and denervation, in which it occurs and often can be extremely debilitating. The method is also useful in conditions. . .

DRWD FIG. 1 is a graphic representation of the results of fractionation of extracts from rabbit skeletal **muscle** fraction II by mono-Q anion exchange chromatography. Subsequent analysis focused on peak 2 because it was shown, as described herein,. . .

DRWD . . . protease, in which the peak of activity degrading Ub-.sup.125 I-lysozyme from the Superose 6 column was concentrated, and 25 .mu.g **protein** was analyzed.

DRWD FIG. 9 is a graphic representation of the effect of ATP-depletion on **protein** breakdown in denervated and normal soleus **muscles**. These data show that overall proteolysis increases primarily by activation of the ATP-dependent pathway following denervation. Values are the means.+-.the. . . for at least 5 rats in which both sciatic nerves were cut, or for unoperated normal rats. Upper Left: Total **protein** degradation on each day after cutting the sciatic nerve and in normal **muscles** from rats of similar size (60-70 g), Upper Right: Effect of ATP-depletion on rates of proteolysis. Lower Left: The relative changes in total **protein** breakdown and in the energy-independent proteolytic process after denervation (i.e., the difference in means rates of proteolysis between denervated **muscles** and normal ones). Lower Right: The relative changes in the ATP-dependent process after denervation.

DRWD FIG. 10 is a graphic representation of the effects of fasting and refeeding on **protein** breakdown in rat extensor digitorum longus **muscle**. Left panel: Total **protein** breakdown and the energy independent process in **muscles** from fed or fasted rats were measured at different times after removal of food and 24 hours after refeeding. Right panel: The ATP-independent component of **protein** breakdown. Values are the means.+-.the SEM for 6 rats.

DRWD FIG. 11 shows results of Northern blot analysis Ub mRNA in **muscle** from fasting and fasted-refed rats. Shown are levels of polyUb mRNA in 10 .mu.g of total RNA/lane isolated from soleus **muscle** of fed rats (a) and fasted rats for 24 hrs. (b) 48 hrs. (c) or fasted 48 hrs. and refed. . .

DRWD FIG. 12 is a graphic representation of levels of total mRNA determined by dot blot analysis in soleus **muscles** of fasted and fasted-refed rats, as described in Example 6. Significant difference from fed animals, *p<0.005, **p<0.05.

DETD The present invention is based on the identification of the pathway responsible for the excessive **protein** degradation which occurs in conditions or disease states in which there is severe loss of body mass (e.g., cachexia) and. . . of constituents of this pathway, which make it possible to inhibit the pathway and the negative nitrogen balance in these **catabolic** states.

DETD As described herein, work undertaken to learn which of the proteolytic systems is responsible for the large increase in **protein** breakdown in skeletal **muscle** during denervation atrophy, fasting and other **catabolic** states (e.g., fever) has shown that most of the accelerated proteolysis in **muscle** in fasting or denervation atrophy is due to activation of the nonlysosomal (cytosolic) ATP-ubiquitin-dependent proteolyte process, which until now has been generally believed to be a constitutive process (often termed

"basal **protein** breakdown") and to be primarily responsible for the elimination of abnormal or short-lived regulatory polypeptides. As described herein, however, it has been shown that there is a specific cellular response which leads to loss of **muscle** **protein** and is triggered by a variety of physiological and pathological stimuli. For example, in fasting, the enhancement of **muscle protein** breakdown requires glucocorticoids and low insulin and in febrile infections, requires interleukin-1 and TNF. As is also described herein, ubiquitin is critical in enhancing the activity of the nonlysosomal ATP-dependent process in **muscle** in denervation atrophy, fasting, and treatment with hormones or endotoxin.

DETD It is possible that multiple steps in the ATP-Ub-dependent pathway are affected in **muscle** by fasting and denervation, but the work described herein has resulted in isolation of a new, rate-limiting component in the large (1500 kDa) enzyme complex which hydrolyzes cell **protein** which are marked for degradation by covalent linkage to the cofactor ubiquitin. Thus, the work described herein has identified a

key target for inhibition. As described, a protease has been identified in **muscle** and has been shown to play an essential role in the cytosolic ATP-ubiquitin-dependent proteolytic pathway now known to be activated in various forms of **muscle** wasting. As further described, a polypeptide inhibitor of the proteasome's degradative activities has also been identified.

DETD . . . present invention relates to a method of inhibiting (reducing or preventing) the accelerated or enhanced proteolysis which occurs in atrophying **muscles** and is now known to be due to activation of the nonlysosomal ATP-requiring process in which ubiquitin plays a critical. . . is inhibited by interfering with the ATP-Ub-dependent pathway at one or more possible steps (e.g., by reducing ubiquitin conjugation of **proteins**, by interfering with activity of UCDEN, or by interfering with activity of one of its components, such as

the novel. . .

DETD The present invention also relates to the discovery in **muscle** of the protease which requires ATP hydrolysis for function and has an essential role in the cytosolic ATP-ubiquitin-dependent proteolytic pathway activated in various forms of **muscle** wasting. This proteolytic enzyme, called "multipain", is a 500 kDa multimer or **protein** complex which appears to be a thiol protease related to the papain family of proteases. It contains 6 or more high molecular weight subunits (50-30 kDa in size) and has been shown to degrade ubiquitin-conjugated **proteins** preferentially, by an ATP-dependent reaction. A variety of observations, also described herein, indicate that this protease is the rate limiting component in the recognition and degradation of **proteins** conjugated to ubiquitin. Multipain also has the ability to depolymerize the multiple-ubiquitin chain by an isopeptidase activity. It is sensitive. . .

DETD Thus, inhibition of the ATP-ubiquitin-dependent pathway is a new approach for treating the negative nitrogen balance in **catabolic** states. This can be effected, for example, through use of an inhibitor of the newly discovered proteolytic enzyme, resulting in reduction of loss of **muscle** mass in conditions in which it occurs. Such an inhibitor can also be used in reducing the activity of the cytosolic ATP-ubiquitin-dependent proteolytic system in cell types other than **muscle** cells. Excessive **protein** loss is common in many types of patients, including individuals with sepsis, burns, trauma, many cancers, chronic or systemic infections,. . . in individuals

receiving cortico-steriods, and those in whom food intake is reduced and/or absorption is compromised. Moreover, inhibitors of the **protein** breakdown pathway could possibly be valuable in animals (e.g., for combating "shipping fever", which often leads to a major weight. . .

DETD The following is a description of the work which led to the discovery that most of the accelerated proteolysis in **muscle** in these conditions is due to activation of the nonlysosomal ATP-requiring process; isolation and characterization of the protease multipain; its. . . of identifying multipain inhibitors and inhibitors identified by these methods and a method of inhibiting multipain and its effect on **muscle** degradation.

DETD Demonstration That the Cytosolic ATP-Dependent Proteolytic Pathway is Critical in Atrophy of Skeletal **Muscle**

DETD As described herein, particularly in Examples 3-5, assessment of whether the accelerated proteolysis evident in atrophy of skeletal **muscles** upon denervation or fasting is catalyzed by the nonlysosomal ATP-dependent or energy-independent degradative systems has been carried out. This work has clearly demonstrated a link between the nonlysosomal ATP-dependent pathway and **muscle** wasting. As described herein, it has been shown that in a variety of **catabolic** states (e.g., denervation, fasting, fever, certain endocrinopathies or metabolic acidosis) **muscle** wasting is due primarily to accelerated **protein** breakdown and, in addition, that the increased proteolysis results from activation of the cytosolic ATP-ubiquitin-dependent proteolytic system, which previously had been believed to serve only in the rapid elimination of abnormal **proteins** and certain short-lived enzymes. The discovery that this pathway is responsible for the accelerated proteolysis in these **catabolic** states is based on studies in which different proteolytic pathways were blocked or measured selectively in incubated **muscles**, and the finding of increased mRNA for components of this pathway (e.g. for ubiquitin and proteasome subunits) and increased levels of ubiquitin-**protein** conjugates in the atrophying **muscles**. As described herein, simple animal models that closely mimic these **catabolic** states (e.g., disuse, atrophy, sepsis, endotoxin-treatment, which mimics fever and muscular dystrophy) have been developed, as have methods for precise measurement of rates of **protein** breakdown in **muscles** during in vitro incubations.

DETD Results showed that when normal skeletal **muscles** incubated in vitro were depleted almost completely of ATP, **protein** breakdown decreased by 40-70%. The ATP-dependent (nonlysosomal) proteolytic process was found to be measured specifically and reproducibly if the residual ATP-dependent process was subtracted from the total **protein** breakdown seen in the control contralateral **muscle**. Within 1 and 3 days after denervation of the soleus, this ATP-dependent process increased by 50-250%, while the residual (energy-independent) process did not change. The rise in this ATP-dependent, nonlysosomal process accounted for all of the increased **protein** breakdown during denervation atrophy, including the rapid degradation of actin (as shown by increased 3-methylhistidine production). This response again accounted for most of the enhanced **protein** breakdown in fasting.

DETD After food deprivation, ATP-dependent proteolysis in the **muscles** increased selectively by 150-350%. After refeeding, this process returned to control levels within 1 day. In addition, in **muscle** extracts from fasted rabbits, the ATP-dependent degradation of

endogenous **proteins** and .sup.14 C-casein was about 2-fold faster than in extracts from fed animals. Similarly, selective increase in ATP-dependent proteolysis in **muscles** occurred in sepsis, as modeled by the injection of endotoxin (LPS).

DETD Thus, as shown herein, the increase in the ATP-dependent process in **muscle** is a specific cellular response, activated in a variety of **catabolic** states, which appears responsible for most of the accelerated proteolysis in atrophying **muscles**. The conditions which influence the ATP-requiring degradative system include denervation atrophy, fasting, fever, certain endocrinopathies and acidosis.

DETD Activation of the ATP-Ubiquitin-Dependent System in **Muscle** During Fasting and Denervation Atrophy

DETD As described above, activation of an ATP-dependent proteolytic process appears responsible for most of the increased **protein** degradation in skeletal **muscle** during fasting and denervation atrophy. Because this process might involve the activation of the ATP-ubiquitin-dependent pathway, the levels of mRNA for ubiquitin (Ub) and Ub **protein** content in such atrophying **muscles** were measured (See Example 6). After food deprivation of rats for 1 day, a 2- to 4-fold increase in the levels of two polyUbiquitin transcripts (2.4 and 1.3 kDa) was detected in the soleus and extensor digitorium longus **muscles**, although their total RNA and total mRNA content fell by 50%. After denervation of the soleus, a 2- to 3-fold.

.

. Ub mRNA upon fasting or denervation was accompanied by a 60-90% rise in the total content of ubiquitin in these **muscles**. When fasted animals were refed, the levels of Ub mRNA in their **muscles** returned to control levels within 1 day.

DETD As discussed above, degradation of many **proteins** in eukaryotic cells involves their conjugation to a small polypeptide, ubiquitin, by an ATP-requiring process. UCDEN (Ub-Conjugate Degrading Enzyme or megapain) degrades the ubiquitinated **proteins**. The precise nature of UCDEN is unclear, although it has been shown that the 1000-1500 kDa (26S) complex can be. . .

DETD As described below, a new type of protease has been identified in skeletal **muscle** and shown to be part of the UCDEN complex. The new protease, multipain, forms a complex of approximately 1500 kDa. .

.

DETD a) by itself degrades ubiquitinated **proteins** in an ATP-dependent process and has little or no activity against typical proteasome substrates, such as N-succinly-Leu-Leu-Val-Tyr-7-amino-4-methyl-coumarin (sLLVT-MCA) and casein;

DETD The new protease has also been shown to degrade nonubiquitinated **protein** (e.g., lysozyme) by an ATP-dependent process, although at a slower rate than it degrades ubiquitinated **protein** (ubiquitinated lysozyme), and to degrade oxidant-damaged hemoglobin by an ATP-independent mechanism. The new protease has been shown to play a critical role in the key cytosolic (nonlysosomal) **protein** degradative pathway and to function synergistically with the proteasome (as a constituent of a complex comparable in size to UCDEN) in the ATP-dependent degradation of ubiquitinated **proteins**. In the large complex, multipain appears to catalyze initial cleavages of ubiquitin-conjugated **proteins**. Taken together, the findings presented herein indicate that multipain is the rate-limiting component in the recognition and degradation of ubiquitin-conjugated **proteins**.

DETD As described in detail in Example 1, the new protease has been obtained from mammalian skeletal **muscle**. Briefly, **muscles**

were obtained and processed, as described in Example 1, in order to isolate the fraction which included the activity degrading Ub-**protein** conjugates. The activity-containing fraction was further separated by chromatography into two peaks with Ub-**protein** (Ub-.sup.125 I-lysozyme)-degrading activity. Peak 2 was shown to account for most of the ATP-stimulated breakdown of ubiquitinated lysozyme and to. . .

DETD . . . Ub-lysozyme. This suggests that a single type of active site is involved in the hydrolysis of these different types of **proteins** .

DETD Assessment of whether ubiquitinated and non-ubiquitinated **proteins** are bound to the same site on the new protease was carried out (Example 1). Results failed to demonstrate competition. . . degradation of Ub-.sup.125 I-lysozyme). This suggests that the new protease has specific binding domains which recognize both ubiquitinated and nonubiquitinated **protein** substrates.

DETD . . . similar or identical to the 1500 kDa Ub-conjugate degrading enzyme, or 26s proteolytic complex UCDEN, isolated previously from reticulocytes and **muscle**. These structures are of similar sizes, are labile, and are activated by the same nucleotides. They degrade the same substrates. . .

DETD . . . complex. The findings described herein also show that the proteasome and multipain function synergistically in the ATP-dependent degradation of ubiquitinated **proteins**. For example, as described in Example 2, when multipain alone degraded Ub-.sup.125 I-lysozyme, the only I product was a peptide. . .

DETD . . . of the proteasome, which inhibits the proteasome's proteolytic activities has been purified from reticulocytes and shown to be an ATP-binding **protein** whose release appears to activate proteolysis. The isolated inhibitor exists as a 250 kDa multimer and is quite labile (at. . . the inhibitor plays a role in the ATP-dependent mechanism of the UCDEN complex. It is possible, for example, that during **protein** breakdown, within the 1500 kDa complex ATP hydrolysis leads to functional release of the 40 kDa inhibitor, temporarily allowing proteasome activity, and that ubiquitinated **proteins** trigger this mechanism.

DETD The purified factor has been shown to inhibit hydrolysis by the proteasome of both a fluorogenic tetrapeptide and **protein** substrates, as described in Example 7. When the inhibitor, the proteasome and partially purified CF-1 were mixed in the presence. . .

DETD . . . the physiological roles of the soluble ATP-Ub-dependent pathway, which is generally believed to be a constitutive process (often termed "basal **protein** breakdown") and to be primarily responsible for the elimination of abnormal or short-lived regulatory polypeptides. As shown herein for the. . . mass and negative nitrogen balance characteristically seen in many disease states or conditions is the result of accelerated or excessive **protein** degradation carried out via this pathway. The **muscle** wasting which occurs upon denervation, fasting, fever or metabolic acidosis is due mainly to this accelerated **protein** breakdown. Now that the responsible pathway and key constituents (e.g., multipain and a natural proteasome regulator) have been identified, it is possible to reduce or abolish the

accelerated **protein** breakdown and, thus, the loss of body mass and the negative nitrogen balance. Multiple steps in the ATP-Ub-dependent pathway may be affected in **muscle** by fasting and denervation, but one clear point of regulation is the rate of production of Ub mRNA, as shown in Example 6. In addition, increased conjugation of **muscle proteins** to ubiquitin has been shown under these conditions.

DETD . . . can serve as the basis for effective methods for reducing this proteolytic process and, thus, combatting negative nitrogen balance and **muscle** wasting in such conditions as cachexia associated with diseases including various types of cancer and AIDS, febrile infection, denervation atrophy. . . inhibition of the ATP-ubiquitin-dependent pathway is an approach to treatment. This results in reduction (total or partial) of the accelerated **protein** breakdown which occurs in numerous physiological and pathological states, but does not affect normal degradative processes carried out via this. . .

DETD . . . play a critical role in the cytosolic proteolytic pathway which has been shown to be activated in various forms of **muscle** wasting. The availability of purified multipain of the present invention makes it possible to define the enzyme's active site or. . .

DETD . . . a key participant and whose activation, as shown for the first time herein, is responsible for most of the increased **protein** degradation which occurs in skeletal **muscle** during fasting, denervation and infection. Inhibitors can be produced which interact specifically with a particular subunit or polypeptide which is. . .

DETD . . . is intended to include DNA encoding the purified multipain obtained as described, DNA encoding a multipain subunit, DNA encoding a **protein** or polypeptide which has substantially the same activity and functional characteristics as those of the purified multipain obtained as described. . .

DETD . . . described herein, Ub mRNA levels increase (i.e., the polyUb gene is specifically induced) under conditions where there is enhanced ATP-dependent **protein** degradation (e.g., atrophying **muscle**, fasting). These levels return to normal when the enhanced degradation is reversed (e.g. , by refeeding). An appropriate oligonucleotide probe. . . and determine whether it is present in greater than normal quantities. This can be used as an indicator of accelerated **protein** degradation.

DETD . . . inhibitor to interfere with activity of the protease. For example, a potential inhibitor can be combined with multipain, a ubiquitinated **protein** substrate (e.g., ubiquitinated lysozyme), ATP and Mg.sup.2+, under conditions appropriate for the protease to degrade the ubiquitin-**protein** conjugate. A control which includes the same components except for the potential inhibitor is used for comparative purposes. Inhibitors are. . .

DETD . . . inhibitors, as well as proteasome inhibitors and UCDEN inhibitors, can be used to reduce (totally or partially) the nonlysosomal ATP-dependent **protein** degradation shown to be responsible for most of the increased **protein** degradation which occurs during fasting, denervation or disuse (inactivity), steroid therapy, febrile infection and other conditions. As described herein, cystatin. . .

DETD . . . be necessary to determine whether any inhibitors found to be effective against the 1500 kDa proteolytic complex can selectively inhibit **protein** breakdown in intact cells. This can be done as

follows: First, crude extracts of **muscle** will be used to test the inhibitor's ability to block the entire ATP-ubiquitin-dependent pathway. Such studies can use model radioactive substrates as well as endogenous cell **proteins**, whose degradation can be easily followed by measuring the appearance of free tyrosine. I. C. Kettelhut, et al., Diabetes/Metab., Rev. 4:751-772 (1988); M. Tischler, et al., J. Biol. Chem. 257:1613-1621 (1982). Promising agents are then tested on intact rat **muscles** and cultured cells, in order to evaluate their efficacy against the intracellular proteolysis, their ability to permeate mammalian cells, and. . . .

DETD . . . for their ability to inhibit the ATP-ubiquitin-dependent degradative process is to do so in cultured cells in which a short-lived **protein** whose degradation is ubiquitin-dependent is produced. Inhibition of the process leads to accumulation of the **protein** in the cytosol. The extent to which the **protein** accumulates in the cytosol can be determined, using known methods. For example, a potential inhibitor of the process can be. . . . potential inhibitor being tested. Cultured cells, such as COS cells, which are stably transformed with a gene encoding a short-lived **protein** whose degradation is ubiquitin-dependent (e.g., a short-lived enzyme, such as a mutant .beta.-galactosidase with an abnormal amino terminus which marks. . . .

DETD If a substance which blocks **protein** synthesis is added to such cells, the enzymatic activity and antigen (**protein**) disappear equally rapidly, making it possible to confirm the potential inhibitor's actions on proteolysis. Measurement of cell growth, ATP content and **protein** synthesis in such cells makes it possible to identify (and avoid) highly toxic substances, which is useful because any agent. . . .

DETD . . . would also be informative to use pulse-chase isotopic methods to follow the rates of breakdown of endogenous short-lived and long-lived **proteins**, especially long-lived **proteins**, especially ones known to be degraded by the ubiquitin dependent pathway (e.g., the oncogene products myc or fos).

DETD Any effective inhibitors are then tested in vitro in incubated rats. In such experiments, the soleus or extensor digitorum longus **muscles** from one leg can be incubated with an inhibitor, while the contralateral, identical **muscle** serves as a control. The great advantage of such approaches is that they are highly sensitive, inexpensive, and do not. . . . al., J. Biol. Chem., 265:8550-8557 (1990). With experience, it is easy,with six animals to demonstrate statistically significant changes in overall **protein** breakdown or synthesis as small as 10-15%. It can be calculated from the average turnover time of **muscle proteins** that even changes of this magnitude in proteolysis could be of therapeutic benefit; if maintained for 2 weeks, a 15% reduction in proteolysis by itself should lead to at least a doubling of mass of a denervated **muscle**. Also of interest will be to follow the effects of the inhibitor on breakdown of myofibrillar **proteins**, which constitutes 60% of the **muscle** mass, and represent the major **protein** reserve in the organism. These **proteins** are lost differentially upon denervation or fasting. K. Furuno, et al., J. Biol. Chem., 265:8550-8557 (1990). The degradation of myofibrillar components can be followed specifically by measuring 3-methylhistidine release from **muscle proteins**, which is a specific assay for breakdown of actin. K. Furuno, et al., J. Biol. Chem., 265:8550-8557 (1990); B. B. Lowell, et al., Biochem. J., 234 (1986). It will be of

particular importance to carry out such studies with **muscles** undergoing denervation (disuse) atrophy or ones from fasted or endotoxin-treated (febrile) animals. In such tissues, overall **protein** breakdown is enhanced, and thus they closely mimic the human disease, but can be studied under well-defined in vitro conditions.. . .

DETD Inhibition of the **protein** degradative process will be useful in a wide variety of conditions in which **muscle** wasting occurs and exacerbates the effects of the underlying condition, further weakening the affected individual. Such conditions include cancer, AIDS, **muscle** wasting after surgery or injury (due to immobilization of the individual or a limb), infection, cachexia due to any cause,. . .

DETD . . . administered to counter weight loss which occurs in animals or to act as growth promoters. Since they act to inhibit **protein** breakdown they should promote net **protein** accumulation and make **protein** synthesis more efficient in growth promotion. For example, they can be administered to animals in order to avoid the epidemic loss of **muscle** mass (net **protein** degradation), referred to as shipping fever, that generally occurs when sheep or cattle are immobilized or confined, such as during. . .

DETD . . . another multipain inhibitor or an inhibitor of another pathway (e.g., a lysosomal or Ca.sup.2+ -dependent pathway) responsible for loss of **muscle** mass.

DETD . . . and .O.sub.2 radicals generated by .sup.60 Co irradiation at a concentration of 50 nmol of oxygen radicals per nmol of **protein** . Davies, K. J. A. J. Biol. Chem., 262:9895-9901 (1987). Casein and lysozyme were radiolabelled with .sup.14 C-formaldehyde and .sup.125 I,. . .

DETD New Zealand white rabbits (4-5 kg) were killed by asphyxiation with CO.sub.2 and the psoas **muscles** were rapidly excised. The **muscles** were trimmed of fat and connective tissue, and then ground on a prechilled meat grinder. Approximately 250 g of **muscle** (wet weight) were suspended in ice-cold buffer (3 ml/per g of tissue) containing 20 mM TRIS-HCl (pH 8.0), 1 mM. . .

DETD . . . column equilibrated in 20 mM TRIS-HCI (pH 7.0) and 1 mM DDT (buffer A). The column was washed until no **protein** was detected in the eluate, and the bound **protein** (Fraction II), which contains most of the ATP-dependent proteolytic activity, was eluted with buffer A containing 0.5M NaCl. The eluted **proteins** ' (Fraction II)-were submitted to ammonium sulfate fractionation.

DETD In order to remove the free proteasome from other activities, **muscle** fraction II was brought to 38% saturation and stirred for 45 min. The insoluble **proteins** were isolated by centrifugation at 10,000.times.g for 20 min, and the 0-38% pellet was then suspended in 20 mM TRIS-HCl. . .

DETD . . . 200 p1 containing 50 mM TRIS-HCl (pH 7.8), 10 mM MgCl.sub.2, 1 mM DTT, and 5 .mu.g of the radioactive **proteins**, 0.5 pg of Ub-conjugates, or 0.5 mM of the fluorogenic peptide. For assays of proteolysis, the reaction mixtures contained approximately 15,000 cpm of Ub-lysozyme or labeled **proteins**. Degradation of 1I-lysozyme, Ub-.sup.125 I-lysozyme, 14C-casein, .sup.14 C-hemoglobin and OH/O.sub.2 treated .sup.14 C-hemoglobin were assayed by measuring the production of. . .

DETD **Proteins** were analyzed by SDS-PAGE (10% polyacrylamide gels), as described by Laemmli. Laemmli, U. K. Nature (London) 227:680-685

(1970). The gel. . .

DETD Immunoprecipitations were performed-by incubation of anti-proteasome IgG (100 .mu.g) with **protein** A-Sepharose, as previously described. Matthews et al., Proc. Natl. Acad. Scii. USA 86:2597-2601 (1989). Control immunoprecipitations were performed using Hyclone. . . rabbits by T. Edmunds and A. L. Goldberg. Matthews et al., Proc. Natl. Acad. Sci. USA, 86:2597-2601 (1989). For immunoblotting, **proteins** were electrophoresed on a 10% SDS-poly-acrylamide gel. After transferring the **proteins** to nitrocellulose sheets, (Hershko et al., Proc. Natl. Acad. Sci. USA, 77:1783-1786 (1980)) immunoblots were performed as previously described. Hough. . .

DETD . . . Chem. 261:2400-2408 (1986), Hough, R., and Rechsteiner, M. J. Biol. Chem. 261:2391-2399 (1986)) but using liver extracts. Although this ubiquitinated **protein** was degraded only slowly in crude extracts, fraction II (the fraction that binds to DEAE-cellulose and contains the ATP-dependent degradative. . . in the absence of Mg.sup.2+ (and in the presence of 1 mM EDTA) did not stimulate the degradation of Ub-conjugated **proteins**.

DETD TABLE I

PURIFICATION SCHEME FOR THE 500 kDA PROTEASE FROM RABBIT SKELETAL **MUSCLE** WHICH DEGRADES UBIQUITINATED LYSOZYME

Fraction	Total **protein** (mg)	Specific activity (cpm/h .times. mg) +ATP	-ATP	ATP stimulation +ATP/-ATP
Crude extract	17433	82	74	1.1
DE52 eluate (Fraction II)	1170	779	338	2.3
0-38% (NH.sub.4).sub.2. . .				

DETD . . . et al., J. Biol. Chem. 262:2451-2457 (1987), Driscoll, J., and Goldberg, A. L. J. Biol. Chem. 265:4789-4792 (1990). The pelleted **proteins** were resuspended, dialyzed, and chromatographed on a column using Mono Q-FPLC (Pharmacia). Two peaks with Ub-.sup.125 I-lysozyme-degrading activity were found. . . megapain complex. However, it is noteworthy that this structure degrades non-ubiquitinated lyxozyme perhaps as readily as it degrades the Ub-conjugated **protein**.

DETD . . . Thus, in its Mr (600KDa) and ability to hydrolyze sLLVT-MCA, peak 4 resembles the proteasome, but it did not degrade **proteins** (lysozyme, casein or hemoglobin) for reasons that are uncertain.

DETD Due to difficulties in preparation of large amounts of Ub-conjugated **proteins**, the concentration of ubiquitinated lysozyme used in the standard assays was about 10 times lower than that of free lysozyme. . .

DETD . . . of the new enzyme when assayed against lysozyme, Ub-lysozyme, or oxidant-treated hemoglobin, although these treatments quantitatively precipitated the purified rabbit **muscle** proteasome, as assayed with .sup.14 C-casein or sLLVT-MCA (Table II). (These various proteasome activities are not directly inhibited by the antibodies, but in these

experiments, these activities were removed together by precipitations with **protein** A-Sepharose). The absence of cross-reactivity between these two multimeric proteases was confirmed by Western blot, where these monoclonal or polyclonal. . .

DETD Table III presents the effects of nucleotides on the degradation of Ub-.sup.125 I-lysozyme by the new activity from skeletal **muscle** . In these assays, the active peak from the Suparose 6 chromatography was incubated with Ub-.sup.125 I-lysozyme at 37.degree. C. for. . .

DETD TABLE III

EFFECT OF NUCLEOTIDES ON THE DEGRADATION OF Ub-.sup.125 I-LYSOZYME BY THE NEW ACTIVITY FROM SKELETAL **MUSCLE**

Compound	Relative activity (%)
None	100
ATP	743
ADP	113
AMP	130
AMP-PNP	90
ATP-.gamma.-S	103
CTP	373
GTP	435
UTP	108
PPi	118

DETD . . . requirement for ATP could also be satisfied in part by CTP or GTP, which caused approximately a 4-fold stimulation of **protein** breakdown (Table III). This nucleotide requirement thus resembles prior findings for the nucleotide specificity for Ub-conjugate degradation by the 1500. . . the proteasome, in which any nucleotide triphosphate, including nonhydrolyzable analogs, could activate hydrolysis of peptide substrates, but the stimulation of **protein** breakdown was only seen with ATP.

DETD . . . to be physiological, and this K.sub.m is consistent with earlier observations on cultured cells, where depletion of cellular ATP blocks **protein** breakdown only when ATP levels are reduced drastically (>75%).

DETD TABLE IV

EFFECT OF INHIBITORS ON DIFFERENT ACTIVITIES OF THE NEW PROTEASE AND ON THE PROTEASOME FROM SKELETAL **MUSCLE**

	NEW PROTEASE			PROTEA-SOME
			OH/O.sub.2 - treated	SLLVT-
Addition	Ub-lysozyme	lysoyzme	hemoglobin	MCA
	Relative activity (%)			
None	100	100	100	100
DFP (1. . .				

DETD The new protease was incubated at 37.degree. C. for 1 h with the **protein** substrates and 2 mM ATP. The proteasome obtained by Superose 6 chromatography was incubated with sLLVT-MCA. Mixtures were preincubated for. . .

DETD . . . A similar effect of cystatin was previously reported for the

ATP+Ub-dependent proteolysis against the very large UCDEN complex from rabbit **muscle**. The inhibition by Stefin A is physiologically interesting, since homologous **protein** inhibitors are present in many mammalian tissues. At similar concentrations, cystatin B showed a 55% inhibition, and no significant effect. . .

DETD . . . seems most likely that a single type of active site is involved in the hydrolysis of these different types of **proteins**.

DETD . . . cystatin and other inhibitors to reduce the degradation of Ub-conjugates correlated with their ability to inhibit breakdown of the other **proteins**. The simplest interpretation of these data would be that all three substrates are degraded by a single active site or. . .

DETD To test if ubiquitinated and nonubiquitinated **proteins** were bound to the same site, the purified enzyme was incubated for 1h at 37.degree. C. in the presence of. . . 0.5 .mu.g of lysozyme), even though the nonlabelled lysozyme and oxidized hemoglobin decreased linearly the breakdown of the homologous radioactive **proteins**. In addition, no competition was detected between lysozyme and oxidant-treated hemoglobin at these concentrations. This failure to demonstrate competition between those 3 substrates suggests that the protease has specific binding domains that recognize these different **protein** substrates and also that Ub-lysozyme breakdown does not involve generation of free lysozyme.

DETD . . . in the presence of ATP. To test this hypothesis, approximately equal amounts of multipain and extensively purified proteasome isolated from **muscle** were incubated at 37.degree. C., with or without Mg.sup.2+ -ATP. Active peaks (1 mg **protein** each) obtained after Superose 6 gel filtration were incubated together in the presence of 1 mM Mg ATP for 30. . .

DETD Fractionation of **muscle** extracts- The psoas **muscles** were excised from New Zealand White (4-5 kg) male rabbits (Millbrook Farms, Mass.), and post-mitochondrial extracts were prepared and fractionated on DEAE-cellulose, as described in Example 1. The **proteins** absorbed to DEAE-cellulose and eluted with 0.5M NaCl (Fraction II) were subjected to (NH4)2SO4 fractionation in order to separate the. . .

DETD . . . 200 .mu.l containing 50 mM TRIS-HCI (pH 7.8), 10 mM MgCl.sub.2, 1 mM DTT, and 5 .mu.g of the radioactive **proteins**, 0.5 .mu.g of .sup.125 I-lysozyme conjugates or 0.5 mM of the fluorogenic peptide, succinly-Leu-Leu-Val-Try-7-amido-4-methylcoumarin (sLLVTA-MCA). The amount of Ub-conjugates was. . .

DETD **Protein** was assayed by the method of Bradford. (Bradford, M. M., Anal. Biochem. 72:248-254 (1976)). **Proteins** were analyzed by SDS-PAGE (10% polyacrylamide) using the method of Laemmli (Pickart, C.M. et al., Arch. Biochem. Biophys. 272:114-121 (1989)). . .

DETD In addition to degrading Ub-lysozyme, the 1500 kDa complex degraded a variety of unconjugated **protein** substrates, as do multipain and the proteasome (Table V, FIG. 7).

DETD . . . 0

Complex	11	92	31

Activity	OH/O.sub.2.sup.- -treated Hemoglobin	Hemoglobin	sLLVT-MCA (units)
Proteasome	1	30	133

Multipain	0.4	7	5
Complex	0.7	29	162

*All **protein** substrates were at 25 .mu.g/ml except Ub.sup.125 Ilysozyme,
which was present at 2.5 .mu.g/ml. (For .sup.125 IUb-lysozyme, this concentration refers. . .

DETD . . . by 65%. A similar inhibition by cystatin of ATP-Ub-dependent proteolysis was previously reported for the UCDEN complex isolated from rabbit **muscle**. Fagan, J. M., et al., Biochem. J., 243:335-343 (1987). Other inhibitors of thiol proteases, like leupeptin or E64, did not. . .

DETD . . . ATP could also be satisfied in part by CTP or GTP, which caused
approximately a 3- to 4-fold stimulation of **protein** breakdown (Table VII). The nucleotide-specificity of the complex resembles prior findings for the nucleotide-specificity for UB-conjugate degradation by reticulocyte extracts,. . . with the activation of the isolated proteasome, which only occurs with ATP and thus probably involves a distinct nucleotide binding **protein**.

DETD . . . ATP would appear to be physiologiclly relevant. Furthermore, this Km is consistent with earlier observations on the energy requirement for **protein** breakdown in intact fibroblasts (Gronostajski, R., Pardee, A. B., and Goldberg, A. L., J. Biol. Chem., 260:3344-3349 (1985)), in which nonlysosomal **protein** breakdown fell only when ATP cellular levels were reduced by more than 70% (i.e., from about 3 mM to below. . .

DETD . . . of the three components (CF-2) of the 1500 kDa complex. Recently an ATPase which corresponds to one of the proteasome-associated
proteins of 95-105 kDa and which may regulate proteasome activity within the complex has been purified. Multipain and the larger complex. . . for cystatin-sensitive proteolytic activity. Thus, in addition to degrading ubiquitinated lysozyme to small peptides, isolated
multipain rapidly disassembles multiple ubiquitinated **protein**, releasing free ubiquitin and **protein**.

DETD Within the 1500 kDa complex, the proteasome and multipain appear to act synergistically in the breakdown of Ub-conjugated **proteins**. Both the rate and extent of conjugate degradation were greater with the complex than with equal roles of multipain alone.. . . to function in
an integrated, perhaps processive, manner. The complex yields short oligopeptides, although in vivo and in reticulocyte extracts, **proteins** are digested all the way to free amino acids. Presumably other exopeptidases catalyze the completion of this hydrolytic pathway.

DETD . . . the lag phase also occurs in vivo, it may mean that if a multipain molecule by itself binds a ubiquitin-conjugate, **protein** degradation proceeds very slowly until multipain also interacts with a proteasome and forms the larger, more active degradative complex.

DETD Demonstration of Activation of the Cytosolic ATP-Dependent Proteolytic Pathway in Atrophy of Skeletal **Muscle** Upon Denervation(Disuse)

DETD . . . described in Examples 3 and 4, activation of the nonlysosomal (cytosolic) ATP-independent proteolytic pathway has been demonstrated in
striated (skeletal) **muscle** during denervation atrophy and fasting and has been shown to be responsible for most of the increased **protein** degradation which occurs in both states.

DETD **Muscle** Incubations
DETD . . . young (60-80 g) male Charles River rats, which were given free access to water and Purina Lab Chow. The soleus **muscle** was denervated as described previously (Furuno K. et al., J. Biol. Chem. 265:8550-8557 (1990)) and sham-operated rats used as controls.. . . the sciatic nerve or after withdrawal of food, the rats were killed and the soleus or extensor digitorum longus (EDL) **muscles** were dissected and incubated in vitro, as described previously. Furuno K. et al., J. Biol. Chem. 265:8550-8557 (1990); Baracos, V.. . . al., Am. J. Physiol. 251:C588-596 and Kettlehut, I. C. Am. J. Physiol., in press (1991). After a 1 hour preincubation, **muscles** were transferred to fresh medium, and tyrosine release measured after 2 hours. The Ca.sup.2+ -free Krebs-Ringer bicarbonate buffer used in. . . 4 .mu.g/ml insulin, 0.17 mM leucine, 0.1 mM isoleucine, 0.2 mM valine,
10M
methylamine, and 50 .mu.M E-64. To deplete **muscles** of ATP, they were incubated with dinitrophenol (at 0.1 and 0.5 mM) and 2 deoxyglucose (5 mM) after removal of. . .
DETD To measure overall **protein** breakdown, the release of tyrosine from cell **proteins** was followed under conditions where **protein** synthesis was blocked. The accumulation of 3-methylhistidine was measured to follow the breakdown of myofibrillar **proteins**; 3-methylhistidine is a specific constituent of actin and myosin Goodman, M. N. Biochem. J. 241:121-127 (1987) and Lowell, B. B.. . .
DETD The ATP content of the **muscles** was determined after preincubation with or without metabolic inhibitors, as described previously. Gronostajski, R. et al., J. Biol. Chem., 260:3344-3349. . .
DETD Measurement of ATP-Depletion on Proteolysis in Skeletal **Muscle**
DETD A simple experimental approach to measuring reliably the ATP-dependent system in intact **muscle** in vitro has been developed.
DETD Despite the fact that **muscle** extracts contain the ATP-Ub-dependent system, Matthews, W., et al., Proc. Natl. Acad. Sci. USA, 86:2597-2601 (1989) and Fagan, J. M.,. . . Fagan, J., J. Biol. Chem., 264:17868-17872 (1989), efforts have repeatedly failed to demonstrate a fall in proteolysis upon depleting intact **muscles** of ATP by using metabolic inhibitors. Goodman, M. N., Biochem. J., 241:121-127 (1987). In other cells studied, including fibroblasts, hepatocytes,. . . Goldbert, A.L., and St. Johh, A., Ann. Rev. Biochem., 45:747-803 (1976), agents that block ATP production were
found
to reduce **protein** breakdown by 50-90%. However, when rat leg **muscles** were incubated in normal media (containing Ca.sup.2+) with cycloheximide, dinitrophenol (DNP), and 2-deoxyglucose, **muscle** ATP content decreased by over 90%, yet overall proteolysis increased by 80-200%. Fulks, R., et al., J. Biol. Chem., 250:290-298 (1975). Both the dark soleus and the pale EDL **muscles** showed a similar activation of proteolysis upon ATP-depletion, as did soleus **muscles** following denervation or fasting of the animals for 2 days. This rise in proteolysis was seen even when the **muscles** were incubated under conditions that reduce net **protein** breakdown (i.e., incubation under tension with insulin and amino acids present). Baracos, V. E., and Goldberg, A. L., Am. J. Physiol., 251:C588-596 (1986). Under these conditions, the **muscles** developed rigor, as is typical upon ATP-depletion. A variety of evidence (see below) indicated that this anomalous
activation
of proteolysis was because ATP depletion in **muscle** leads to Ca.sup.2+ entry into the cytosol and activation of Ca.sup.2+ dependent

proteases, and that the resulting stimulation of overall. . .

DETD TABLE IX

EFFECT OF INHIBITORS OF DIFFERENT CELL PROTEASES AND ATP PRODUCTION ON BREAKDOWN OF MYOFIBRILLAR AND TOTAL **PROTEIN** IN DENERVATED SOLEUS

Pathway Inhibited	Total **Proteins** Tyrosine Release (pmol/mg/2h)	(%)	Myofibrillar **Proteins** 3-Methylhistidine Release (pmol/mg/2h)	(%)
None	328 .+-. 10	100	5.11 .+-. 0.21	100
Lysosomal	330 .+-. 11	100. . .		

DETD Values are the means.+-.SEM for 5 **muscles** three days after section of the sciatic nerve. Significant difference, *p<0.1. **Protein** breakdown measured in **muscles** at resting length in Ca.sup.2+ -free Krebs-Ringer bicarbonate buffer containing insulin and amino acids. Methylamine (10 .mu.M) is an inhibitor. . .

DETD Conditions for Measuring ATP-dependent Proteolysis in Incubated **Muscles**

DETD . . . measure the ATP-dependent process, it was necessary to prevent the activation of Ca.sup.2+ -dependent proteases upon ATP-depletion (see above). The **muscles** were therefore maintained at resting length (Baracos, V. E., and Goldberg, A. L., Am. J. Physiol., 251:C588-596 (1986)), in Ca.sup.2+. . . al., Agric. Biol. Chem., 42:523-528 (1978). Prior studies showed that these conditions block the activation of proteolysis in anoxic (shortened) **muscles** (Baracos, V. E. and A. L. Goldberg, Am. J. Physiol., 251:C588-596 (1986); and Kettelhut, I. C. et al., Am. J.. . . al., Am. J. Physiol., 13:E702-710 (1986)). As described previously, in this medium inhibitors of ATP production were found to reduce **protein** breakdown in **muscle** (FIG. 9), as they do in other cells. Gronostajski, R., et al., J. Biol. Chem., 260:3344-3349 (1985) and Goldberg, A. L., and St. John, A., Ann. Rev. Biochem., 45:747-803 (1976). To prevent lysosomal **protein** breakdown in these **muscles** (Furuno K. , and Goldberg, A. L., Biochem. J., 237:859-864 (1986); Zeman, R. J. et al., J. Biol. Chem., 260:13619-13624. . . S. J. Cell. Biol., 90:665-669 (1981). In addition, the E-64c inactivates lysosomal thiol proteases (cathepsins B,H, and L) in intact **muscles**, Baracos, V. E., et al., Am. J. Physiol., 13:E702-710 (1986). These incubation conditions do not affect the levels of ATP or creatine phosphate in the tissues or the rates of **protein** synthesis. Baracos, V. E., et al., Am. J. Physiol. 251:C588-596 and Kettlehut, I. C. Am. J. Physiol., in press (1991).

DETD Even though lysosomal and Ca.sup.2+ -dependent proteolytic systems were blocked, the **muscles** showed linear rates of **protein** breakdown (FIG. 10). These rates were similar to those in **muscles** maintained in complete medium lacking the inhibitors. Baracos, V. E., et al., Am. J. Physiol. 251:C588-596 (1986); Kettlehut, I. C.. . . This finding agrees with prior studies showing that lysosomal and Ca.sup.2+ -dependent processes make a very minor contribution to "basal" **protein** breakdown. Rechsteiner, M.,

Ann. Rev. Cell Biol., 3:1-30 (1987); Dice, J. G., FASEB J., 1:349-356 (1987); Gronostajski, R., et al.,. . . 260:13619-13624 (1985) and Baracos, V. E., and Goldberg, A. L., Am. J. Physiol., 251:C588-596 (1986). When normal soleus or EDL **muscles** in this medium were depleted of up to 96% of their ATP (with dinitrophenol and 2-deoxyglucose), there was a 50-70% reduction in **protein** degradation (FIG. 10), which resembles the fraction of **protein** breakdown that is ATP-dependent in fibroblasts. Gronostajski, R., et al., J. Biol. Chem., 260:3344-3349 (1985). To quantitate this ATP-dependent component, the **muscle** of one limb was depleted of ATP, while the contralateral **muscle** served as a control. The rate of **protein** degradation in the two limbs were compared. The net decrease in overall **protein** breakdown comprises the ATP-dependent component and could thus be measured highly reproducibly in **muscles** in different physiological states (FIGS. 9 and 10). Kettelhut, I. C., et al., Diabetes/Metabolism-Reviews, 4:751-772 (1988); Han, H. Q., et. . .

DETD To deplete **muscles** of ATP, they were preincubated for 1 hour with 2,4-dinitrophenol (DNP) and 2-deoxyglucose to block both oxidative phosphorylation and glycolysis.. . . (1985)), and hepatocytes, Hershko, A., and Tomkins, G. M., J. Biol. Chem., 246:710-714 (1971), these agents block ATP production and **protein** breakdown reversibly. Neither inhibitor affected the ATP-dependent or energy-independent proteolytic systems in cell-free extracts of **muscle**. Typically, preincubation with DNP (0.1 mM) and 2-deoxyglucose (5 mM) for 1 hour reduced ATP content by>85%, and 0.5 mM DNP with deoxyglucose (5 mM) depleted ATP by>96% in normal **muscles**. These treatments caused similar reductions in ATP content in denervated **muscles** and in **muscles** from fasted animals whose initial ATP stores were also similar to those of control **muscles**. These different concentrations of DNP caused a similar reduction in **protein** breakdown. In these ATP-depleted tissues, the residual (energy-independent) **protein** degradation occurred at linear rates for several hours, and the intracellular pools of tyrosine were similar to those in the contralateral (untreated) **muscles**.

DETD Changes in **Protein** Breakdown during Denervation Atrophy

DETD When the sciatic nerve of a rat is cut, the unused soleus **muscle** on that limb undergoes rapid atrophy, losing about 30% of its weight and **protein** content within 3 days. Furuno K., et al., J. Biol. Chem., 265:8550-8557 (1990) and Goldspink, D. F., Biochem. J., 156:71-80 (1976). During this period, overall **protein** breakdown increases and by 3 days is 2- to 3-fold greater than in the contralateral control soleus, Furuno K., et al., J. Biol. Chem. 265:8550-8557 (1990). A similar rise in overall proteolysis was seen when the denervated and control **muscles** were incubated in normal Krebs-Ringer bicarbonate or under conditions which prevent lysosomal or Ca.sup.2+ -dependent proteolysis, Furuno K., et al.,. . .

DETD To test whether the ATP-dependent pathway is responsible for the enhanced **protein** breakdown, the atrophying and control soleus were depleted of ATP at different times after nerve section, as described above. Control experiments showed that neither denervation for 3 days nor fasting affected the **muscle**'s initial ATP content or the decrease in ATP induced with DNP and, deoxyglucose (Table IX). However, depletion of cellular ATP caused a much larger net decrease in

proteolysis in the denervated **muscles** than in controls (FIG. 9). For example, in a typical experiment these inhibitors decreased proteolysis by 53.+-.6 pmol/mg/2 h (43%). . . the residual rates of proteolysis in the denervated and control tissues did not differ (FIG. 9). Thus, in the atrophying **muscles**, a nonlysosomal ATP-dependent proteolytic process seems to be activated, while no change occurs in the residual energy-independent process.

DETD Overall **protein** breakdown in the soleus was enhanced by 1 day after nerve section and then rose progressively during the next 3. . . failure to block completely the ATP-dependent pathway. The rise in the ATP-requiring process could account for all of the increased **protein** breakdown in the denervated **muscle** maintained in this way (FIG. 9).

DETD Demonstration of Activation of the Cytosolic ATP-Dependent Proteolytic Pathway in Atrophy of Skeletal **Muscles** in Fasting

DETD **Muscles** of fasting rats were studied to test whether this degradative process is activated under other physiological conditions where **muscle protein** breakdown rises. In animals deprived of food, there is a rapid increase in **muscle protein** breakdown which appears essential to provide the organism with amino acids for gluconeogenesis. Li, J. B., and Goldberg, A. L.,. . . A. L., et al., Federation Proc., 39:31-36 (1980) and Lowell, B. B., et al., Metabolism, 35:1121-112 (1986). When the EDL **muscles** from fasted animals were incubated under conditions that block lysosomal and Ca.sup.2+ -dependent degradative processes, they showed a large increase. . . (FIG. 10), in accord with observations on 3-methyl-histidine production, Lowell, B. B., et al., Metabolism, 35:1121-112 (1986). However, when the **muscles** from the fasted or fed animals were incubated with metabolic inhibitors to prevent the ATP-requiring process, these differences in their rates of **protein** breakdown were eliminated. Thus, the increase in **muscle** proteolysis in fasting seems to be due to an enhancement of an energy-requiring nonlysosomal process.

DETD . . . evident 1 day after removal of food and could account for all of the increased proteolysis seen in the EDL **muscle** under these incubation conditions (FIG. 12). In fasting, the enhancement of overall proteolysis is greater in the pale **muscles**, such as the EDL, than in the dark soleus. Li, J. B., and Goldberg, A. L., Am. J. Physiol., 231:441-448 (1976). Accordingly, the soleus **muscle** showed a similar, but a smaller, rise in the ATP-dependent process. On the average, the rise in proteolysis in the. . .

DETD Upon refeeding the rats, **protein** breakdown in the EDL decreased back to basal levels within 1 day (FIG. 10). Again, this response was due to. . .

DETD One of the major features of denervation atrophy is differential loss of myofibrillar **proteins**, but the system responsible for their accelerated degradation has not been identified. Furuno K., et al., J. Biol. Chem., 265:8550-8557 (1990). The breakdown of these **proteins** can be followed by measuring 3-methyl-histidine production, which is a specific constituent of actin, and in certain **muscles** of myosin. Goodman, M. N., Biochem. J, 241:121-127 (1987) and Lowell, B. B., et al., Metabolism, 35:1121-112 (1986). When these **proteins** are hydrolyzed, this amino acid cannot be reutilized in **protein** synthesis, and thus its appearance as an indication of myofibrillar **protein** breakdown. Goodman, M. N., Biochem. J, 241:121-127 (1987) and Lowell, B. B., et al., Metabolism,

35:1121-112 (1986). The increased production. . . lysosomal and Ca.sup.2+ -dependent proteolysis. Furuno K., et al., J. Biol. Chem., 265:8550-8557 (1990). These findings and those for overall **protein** breakdown (FIG. 11) indicate that enhancement of a nonlysosomal ATP-dependent process is primarily responsible for the **muscle** atrophy.

DETD Measurement of Proteolysis in **Muscle** Extracts

DETD Psoas **muscles** from fed and fasted rabbits were used to obtain sufficient material for assay of the ATP-dependent system in cell-free extracts.. . . 20% (similar to that seen in rats deprived of food for 1 day). The animals were anesthesized, and their psoas **muscles** dissected and homogenized as described previously. Fagan, J. M., et al., J. Biol. Chem., 261: 5705-5713 (1986).

DETD After centrifugation at 10,000.times.g and then at 100,000.times.g, the **muscle** extracts were fractionated on DE52 cellulose to remove ubiquitin and most cell **proteins**, as described previously. Han, H. Q. et al., Federation Proc, 2:A564 (1988) and Waxman, L., et al., J. Biol. Chem.,. . . containing Tris (50 mM, pH7.8), dithiothreitol (1 mM), and 20% glycerol, and concentrated before assay of activity. Degradation of endogenous **muscle proteins** was assayed by measuring the production of free tyrosine, which was determined fluorometrically after precipitation of **proteins** with trichloroacetic acid. Tischler, M. et al., J. Biol. Chem., 257:1613-1621 (1982) and Fulks, R., et al., J. Biol. Chem.,. . .

DETD . . . the soluble ATP-requiring proteolytic system which involves ubiquitin is activated during fasting or denervation atrophy. However, such measurements on intact **muscles** cannot distinguish other possible changes in these **catabolic** states. Therefore, soluble cell-free extracts of **muscles** from fed and fasted rabbits were used in order to test whether the increased proteolysis in fasting is due to activation of the ATP-Ub-dependent system. Cell-free preparations showing ATP-Ub-dependent proteolysis have been described in extracts of rabbit **muscles**. Fagan, J. M., et al., Biochem. J., 243:335-343 (1987). The proteolytic system from rabbit **muscles** was partially purified by high-speed centrifugation and ultracentrifugation to remove myofibrils and membranous components, and then it was subjected to DEAE chromatography to remove most (>90%) of the soluble **proteins**, including free ubiquitin. The resulting fraction contains all the enzymes for Ub-conjugation and hydrolysis of Ub-**protein** conjugates, Herskho, A., J. Biol. Chem., 263:15237-15240 (1988); Rechsteiner, M., Ann. Rev. Cell-Biol., 3:1-30 (1987); Waxman, L., et al., J.. . .

DETD In these extracts, the hydrolysis of endogenous **proteins** (shown by tyrosine production) increased 5- to 9-fold upon addition of ATP and even further upon addition of ATP with. . .

DETD TABLE X

EFFECTS OF FASTING OF RABBITS ON ATP-UBIQUITIN-ACTIVATED PROTEOLYSIS IN EXTRACTS OF PSOAS **MUSCLE**

Condition	No addition	+ATP	+ATP + Ub
Hydrolysis of Endogenous **Proteins** (nmol try released/2 hr)			
Fed	0.6 .+-. 0.1	5.7 .+-. 0.9	

9.2 .+-. 1.8

Fasted 2.0 .+-. 0.1

10.8 .+-. 2.1

15.2 .+-.. . .

DETD . . . **p<0.01. These assays were performed on partially purified proteolytic fractions ("Fractions II") as further described in the Example. Breakdown of endogenous-**proteins** (tyrosine production) was measured for 2 hours at 37.degree. C. with 5 mg of Fraction II **protein**. Degradation of .sup.14 C-Casein was assayed 37.degree. C. for 1 hour with 400 .mu.g of Fraction II **protein** and 20 .mu.g .sup.14 C-casein. Assays were performed in Tris (50 mM, pH 7.8), dithiothreitol (1 mM), and MgCl (10. . .

DETD To further test for an activation of the ATP-dependent degradative system, rather than an alteration in the endogenous cell **proteins** which served as substrates, .sup.14 C-methyl-casein was used as a substrate (Table X). This **protein** is also degraded rapidly by ATP-independent enzymes, and this ATP-independent process appeared to increase upon fasting (although this trend did. . .

store

large amounts of food in their gastrointestinal tract. However, no such increase in proteolysis was seen in extracts of **muscles** from rabbits deprived of food for shorter periods than 6 days, at which time they showed no weight loss and. . . substrates clearly indicate an increased capacity of the ATP-dependent degradative system in fasting, as suggested by the measurements on incubated **muscles** (FIG. 10).

DETD Further Evidence for Activation of the ATP-Ubiquitin-Dependent Process in Various **Catabolic** States

DETD Activation of the ATP-ubiquitin-dependent proteolytic process was shown to be responsible for most of the increased **protein** degradation in skeletal **muscle** during denervation atrophy, fasting and febrile infection, as described below. In addition, levels of polyubiquitin mRNA and MRNA for proteosome units are shown to increase in skeletal **muscle** during denervation atrophy, fasting and febrile infection, as shown below. Similar data have been obtained in rats with metabolic acidosis (induced by injection with **ammonium chloride**) or suffering with cancer cachexia (induced by a transplantable hepatoma growing in ascites).

DETD **Muscle** preparations

DETD . . . Lab chow and water "ad libitum". All treatments were carried out as described in Example 3. To denervate the soleus **muscles** of one hind limb, the sciatic nerve was cut about 1 cm above the popliteal fossa, while the animals were. . . J. Biol. Chem., 265:8550-8552 (1990). In all cases the animals were killed by cervical dislocation and the EDL and soleus **muscles** were dissected as described in the previous examples.

DETD Total RNA from **muscle** was isolated by the guanidinium isothiocyanate/CsCl method, and electrophoresis of RNA was performed in 1% agarose gels containing 0.2M formaldehyde.. . .

DETD . . . dot blot analysis, four different concentrations (2-fold dilutions from 1.5 .mu.g) of total denatured RNA from the soleus or EDL **muscles** were spotted on Gene Screen membranes. The amount of RNA applied to each dot was maintained at 1.5 .mu.g by adding E. colitRNA (which in the absence or rat **muscle** RNA did not show any hybridization). The hybridization probes were a Ub cDNA fragment

(Agell,

N. et al., Proc. Natl.. . . dot intensities of the autoradiograms by automated densitometric scanning. The unpaired Student's t-test was

used

in statistical analyses to compare **muscle** of fed and fasted

animals and the paired t-test was used to compare contralateral denervated and control **muscles**.

DETD Measurements of total ubiquitin content (which includes both free Ub and Ub ligated to **proteins**) were carried out using the immunochemical method described by Riley D. A. et al., J. Histochem. Cytochem., 36:621-632 (1988).

DETD To test whether the level of Ub mRNA increases when **muscle protein** breakdown rises, the levels of polyUb transcripts in rat **muscles** were determined at different times after food deprivation. As shown in FIG. 11, the soleus contained two transcripts of 2.4. . . . U. in Oxford Surveys on Eukaryotic Genes (Maclean, N., ed.) 4:76-91'(1987)). The levels of both transcripts increased progressively in the **muscles** of fasted animals. The relative levels of Ub mRNA in these tissues of fasting rats were measured by dot-blot analysis. . . . (FIG. 12). After 48 hours of food deprivation, the levels of total Ub mRNA in the extensor digitorum longus (EDL) **muscle** showed a 4-fold increase over **muscles** of control animals (FIG. 13, upper panel). The soleus **muscle**, which atrophies less than the EDL in fasting (Li, J. B. and Goldberg, A. L., Am. J. Physiol, 231:441-448 (1976)),. . . .

DETD . . . . rats were then provided food for 24 hours. By 24 hours of refeeding, the levels of polyUb mRNA in these **muscles** had returned to levels in **muscles** of normal animals. This rise and fall in the amount of polyUb mRNA thus parallels the changes in overall rates of **protein** degradation (FIG. 10) and in the ATP-dependent degradative process (FIG. 10).

DETD . . . . mRNA is regulated in a specific manner in fasting, whether the total amount of RNA or of mRNA in these **muscles** may also have changed after food deprivation in a similar way as polyUb mRNA was assessed. The total RNA content. . . . amount of mRNA (i.e., poly-A-containing RNA) in the soleus and EDL decreased to approximately 50% of the levels found in **muscles** of fed animals. Total RNA fell from 72.+-.3.5 to 35.+-.1.6 ug/**muscle** and total mRNA (expressed in arbitrary densitometric units) from 2133.+-.376 to 1004.+-.20 units/**muscle** in the soleus during fasting. In the fasted EDL, total RNA decreased from 68.+-.6 to 38.5.+-.1 ug/**muscle** and total mRNA from 710.+-.73 to 413.+-.11 units/**muscle**. The ratio of total mRNA to total RNA, unlike Ub mRNA, thus, did not change significantly during the 48 hours. . . .

DETD Subsequent experiments tested whether the increase in polyUb mRNA in fasting is unique to skeletal **muscle** or whether other rat tissues show similar responses 2 days after food deprivation. Enhanced proteolysis in fasting has been attributed. . . . process. In the heart (ventricle) of fasting rats, a rise in polyUb mRNA occurred similar to that seen in EDL **muscle**. By contrast, no such change was seen in any other tissue tested, including liver, spleen, adipose tissue, brain, testes and kidney. In the liver, kidney, and adipose tissue a marked loss of weight and **protein** occurred on fasting, but as expected neither testes nor brain showed significant weight loss under these conditions. Thus, during fasting, the rise in Ub mRNA appears to be a specific adaptation in striated **muscle** and is not seen in other tissues.

DETD A similar 2- to 3-fold acceleration of the ATP-dependent proteolytic process occurs in **muscle** during denervation atrophy. To test whether in this condition the expression of polyUb genes may also be activated, we analyzed. . . . 1 and 3 days following denervation, the

levels of polyUb transcripts increased markedly above the levels in the contralateral control **muscle**. Dot blot analysis of the **muscles** revealed a 2 to 3-fold increase in polyUb mRNA content as a proporation of total mRNA following denervation (Table XI). Although the size of Ub mRNA level of control **muscles** did not change during the course of this study, by contrast the total RNA in-the denervated soleus decreased by 40%. . .

DETD This increase in mRNA for ubiquitin correlated with accelerated proteolysis in the **muscle**.

DETD TABLE XI

EFFECT OF UNILATERAL DENERVATION OF RAT SOLEUS **MUSCLE** ON THE CONTENT OF PolyUb mRNA, TOTAL RNA AND WEIGHT

Time after operation	Control	Denervated	Control	Denervated
Ub mRNA/.mu.g	2.4 .+-. 0.2	6.1 .+-.. . . 1.4	22.7 .+-. 0.8	23.7 .+-. 1.4
RNA Total RNA (.mu.g)/	42.3 .+-. 2.2	36.7 .+-. 1.3**	54.0 .+-. 6.5	32.5 .+-. 4.2*
soleus **Muscle** weight (mg)	28.8 .+-. 0.7	26.3 .+-. 0.8**	30.7 .+-. 1.1	23.5 .+-. 2.1*
Weight loss (% of control)	-9%			-23%*

DETD Ubiquitin Content of the **Muscles**

DETD To determine whether the increase in polyUb mRNA actually resulted in increased production of Ub, the total amount of this **protein** in the **muscles** was quantitated by immunoassay (Table XII). These arrays measured both free Ub and Ub conjugated to cell **proteins**. (Riley, D. A. et al., J. Histochem. Cytochem., 36:621-632 (1988) In EDL **muscles** from animals fasted 2 days, a 63% increase in Ub levels was observed over levels in fed controls. An even larger increase of 91% was seen in the Ub content of soleus **muscles** 2 days after cutting the sciatic nerve. Thus, total Ub content correlated with the increase in ATP-dependent proteolysis and in. . .

DETD The covalent linkage of Ub to cell **proteins** is known to mark them for rapid degradation. Therefore, we also measured the **muscles** content of ubiquitin-**protein** conjugate in normal and denervated **muscle**. As shown in Table XIII, the levels of ubiquitinated **proteins** increased by 158% after denervation for 2 days. A similar increase in ubiquitinated **proteins** was seen upon fasting of the rats (data not shown) and

this difference disappeared upon refeeding the animals for one day. These findings further indicate activation of the ubiquitin dependent process in atrophying **muscles**.

DETD In the denervated **muscle** and in fasted animals, there was also found an increase in rate of proteasome synthesis, as indicated by a 2-3. . . and C-9 and in related experiments, a similar increase was seen in mRNA for three other subunits. Thus, the atrophying **muscles** are increasing levels of multiple components-of this degradative pathway.

DETD . . . mRNA following denervation, fasting or refeeding occur in parallel with and appear to be linked to the alterations in overall **protein** breakdown and in degradation of myofibrillar **proteins** measured in the incubated **muscles**. The rise in Ub MRNA seen in the atrophying **muscles** appears responsible for their increased Ub content (Table XII), which occurred despite the net loss of total **muscle protein**. Furthermore, the preceding examples demonstrated that these changes in overall proteolysis are due to activation of a nonlysosomal ATP-dependent process and that fasting leads to enhanced ATP-Ub-stimulated proteolysis in soluble extracts of **muscle**.

DETD . . . conclusion that the Ub-dependent proteolytic system is enhanced under these conditions. As described herein, it was also observed that the **muscles** from fasting animals and denervated **muscles** also showed higher levels of Ub-conjugated **proteins** and of mRNA encoding the proteasome, which is essential in the breakdown of such ubiquitinated **proteins**. These results together indicate that the Ub-dependent system in **muscle** is precisely regulated by contractile activity and food intake. The response to fasting requires adrenal steriods (Kettelhut, I. C. et. . .

DETD The changes shown here in Ub mRNA levels parallel exactly the changes in overall **protein** degradation and in the breakdown of myofibrillar **proteins**, both of which were shown in the preceding examples to occur by an ATP-dependent nonlysosomal process. The present data thus suggest a more general role for this system in the degradation of normal **muscle proteins**, including probably the long-lived myofibrillar components.

DETD The polyUb gene seems to be an example of a gene that is specifically induced in atrophying **muscles**. In fasting or denervation atrophy, when **muscle** mass and overall RNA are decreasing, the levels of polyUb mRNA and Ub concentration rose. In contrast, the levels of. . . Ub mRNA levels and Ub production seem to be regulated inversely to total RNA or to mRNA for the Ub-extension **protein** .

DETD . . . physiological interest is the finding that the increase in Ub mRNA (and presumably, therefore, in Ub) is restricted to striated **muscle**. Such changes also occur in the rat heart, which in fasting undergoes considerable weight loss. These findings suggest that ATP-dependent proteolysis also rises in cardiac **muscle** under such conditions, presumably by similar mechanisms as in skeletal **muscle**, although systematic studies have not been reported. The absence of any change in Ub levels in testes or brain was anticipated, since the **protein** content and size of these organs are maintained during a fast. However, it is noteworthy that levels of Ub mRNA. . . relative importance of different proteolytic processes

differ between tissues and that the ATP-Ub-dependent pathway is of special significance in striated **muscle**, particularly in **catabolic** states.

DETD TABLE XII

EFFECTS OF DENERVATION AND FASTING ON UBIQUITIN LEVELS IN RAT SKELETAL **MUSCLES**

Muscle	Total **Protein** (mg/**muscle**)	Total Ubiquitin (pmol/**muscle**)	(pmol/mg **protein**)
Soleus			
Innervated	3.5 .+-. 0.4	89 .+-. 5	27 .+-. 2
Denervated	2.7 .+-. 0.2	137 .+-. 12	51 .+-. 2
Difference	-0.8 .+-.. . .		

DETD Values are the means.+-. SEM for extensor digitorum longus (EDL) **muscles** from four fed or fasted animals and for seven paired soleus **muscles** two days following section of one of the sciatic nerves. Significance difference, *p<0.05, **p<0.01.

DETD . . . 2 DAYS ON ATP-DEPENDENT PROTEOLYSIS AND LEVELS OF UBIQUITIN AND UBIQUITIN-CONJUGATES IN RAT SOLEUS

	ATP-Dependent Proteolysis (pmol tyr/mg/2h	Free Ubiquitin	Ubiquitin Conjugates (pmol Ub/mg **protein**)	Total Ubiquitin
Control	63.0 .+-. 11	17.0 .+-. 1.3	10.0 .+-. 0.7	27 .+-. 1.9
Denervated	201.0 .+-. 17	25.0 .+-. 1.2	26.0. . .	

DETD TABLE XV

INJECTIONS OF E. COLI ENDOTOXIN (LPS) RAPIDLY STIMULATE **PROTEIN** BREAKDOWN SIMILARLY IN RAT EXTENSOR DIGITORUM LONGUS **MUSCLE**

Injection	Saline	Treated	Difference
	Proteolysis (nmol tyrosine/rng/2h)		
LPS	0.214 .+-. 0.013	0.280 .+-. 0.015	+31% P < 0.01

INJECTION OF ENDOTOXIN (LPS) ACTIVATES THE ATP-DEPENDENT PATHWAY OF **PROTEIN** BREAKDOWN IN RAT **MUSCLES**

Addition Proteolysis (nmol tyrosine/mg/2h)

Non Lysosomal

0.145 .+-. 0.009
0.190 .+-. 0.017
+31% P < 0.05

Proteolysis*
After ATP 0.094 .+-. 0.004
0.102 .+-.. . .

DETD Activation of **Protein** Breakdown Durin, Systemic Infections

DETD One other condition where **muscle protein** breakdown increases markedly is during systemic infections of bacterial, viral or parasitic origin. Patients with sepsis, which often follows traumatic injuries, tend to be in marked negative nitrogen balance due mainly to accelerated **muscle** pro breakdown. This response is associated with fever and is part of the body's acute phase response. It can be.

.

. released by activated macrophages. As shown in Table XV, 6 hours after endotoxin injection, animals were killed and their leg **muscles** studied in vitro. The EDL showed a rapid increase in overall **protein** breakdown. This response was not due to the lysosomal or calcium activated proteases. When the ATP-dependent degradative system was measured, it had increased by 70% and could account for the overall increase of **protein** breakdown in the animals. Treatment of the rats with endotoxin also caused 2-3 fold increase in the levels of polyUb mRNA in these **muscles** within 6-7 hours. This rise in polyUb mRNA which resembles the response seen in fasting or denervation, was not seen in other tissues. Northern analysis of gastrocnemius **muscles**, excised shows after injection of E. coliendotoxin (40 .mu.g/100 g body weight), using cDNA probes of polyubiquitin genes also showed induction of ubiquitin in RNA (data not shown). These findings thus indicate a common biochemical program in **muscle** leading to enhanced **protein** breakdown in these three **catabolic** states and others, including cancer cachexia as induced in rats carrying Yochida hepatoma in ascities and in rats with metabolic. . .

DETD . . . (Ciechanover, A. et al., Biochem. Biophys. Res. Comm. 81:1100-1105 (1978)). Lysates were then prepared and subjected to DE-52 chromatography. The **protein** eluted with 0.5M KCl (Hershko, A. et al., J. Biol. Chem., 258:8206-8214 (1983)) was concentrated using ammonium sulfate to 80%. . . suspended in 20 mM Tris-HCl (pH 7.6), 1 mM DTT (buffer A). Following extensive dialysis against the same buffer, the **protein** (fraction II) was either stored at -80.degree. C. in 0.5 mM ATP or fractionated further.

DETD . . . for 20 minutes, as described by Ganoth et al. (Ganoth, D. et al., J. Biol. Chem. 263:12412-12419 (1988)). The precipitated **proteins** were collected by centrifugation at 10,000.times.g for 15 minutes. The pellet was resuspended in buffer A and brought again to.

. . buffer, the 0-38% pellet was chromatographed on a Mono-Q anion exchange column equilibrated with buffer A containing 10% glycerol. The **protein** was eluted using a 60 ml linear NaCl gradient from 20 to 400 mM. Fractions which inhibited the peptidase activity. . .

DETD . . . ammonium sulfate precipitations. The supernatants were brought to 80% saturation with ammonium sulfate and mixed for 20 minutes. The precipitated **protein** was collected by centrifugation, resuspended in buffer A, and dialized extensively against this buffer. The proteasome was isolated by Mono-Q. . .

DETD . . . was added. Reactions were carried out at 37.degree. C. for 60 minutes with .sup.125 I-lysozyme or 10 minutes with Suc-LLVY-MCA.

Protein hydrolysis was assayed by measuring production of radioactivity soluble in 10% trichloroacetic acid, and peptide hydrolysis by the release of. . .

DETD These results suggest strongly that the inhibitor corresponds to CF-2 and thus is essential for hydrolysis of Ub-ligated **proteins**. One unusual property of CF-2 is that it is quite labile upon heating to 42.degree. C., but is stabilized by. . .

DETD . . . (1989)). However, a readily apparent band of 40 kDa was evident in this fraction. To further address the question of **proteins** associated with the proteasome, fraction II was immunoprecipitated using and anti-proteasome monoclonal antibody and analyzed by SDS-PAGE. Ub-conjugate degrading activity. . .

CLM What is claimed is:
1. A therapeutic composition for use in the treatment of diseases or conditions characterized by accelerated **muscle** wasting, said composition comprising an inhibitor which is capable of specifically interfering with the functioning of either one or both of the ubiquitin conjugation or proteolysis steps of the non-lysosomal ATP-requiring ubiquitin-dependent proteolytic process in **muscle** cells.

. . . . capable of specifically interfering with the functioning of the ubiquitin conjugation step of the non-lysosomnal ATP-requiring ubiquitin-dependent proteolytic process in **muscle** cells.

. . . . is capable of specifically interfering with the functioning of the proteolysis step of the nonlysosomal ATP-requiring ubiquitin-dependent proteolytic process in **muscle** cells.

L15 ANSWER 41 OF 109 USPATFULL

AB Substituted heterocycles of the general structural formula: ##STR1## are tachykinin receptor antagonists useful in the treatment of inflammatory diseases, pain or migraine, asthma, and emesis.

AN 1998:82754 USPATFULL

TI Morpholine compounds are prodrugs useful as tachykinin receptor antagonists

IN Dorn, Conrad P., Plainfield, NJ, United States
Hale, Jeffrey J., Westfield, NJ, United States
Maccoss, Malcolm, Freehold, NJ, United States
Mills, Sander G., Woodbridge, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5780467 19980714 <--

AI US 1997-907738 19970808 (8)

RLI Division of Ser. No. US 1995-525870, filed on 8 Sep 1995, now patented, Pat. No. US 5691336 which is a continuation-in-part of Ser. No. US 1994-206771, filed on 4 Mar 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Higel, Floyd D.

LREP Thies, J. Eric, Rose, David L.

CLMN Number of Claims: 19

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 7260

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5780467 19980714 <--

SUMM The receptor for substance P is a member of the superfamily of G

protein-coupled receptors. This superfamily is an extremely diverse group of receptors in terms of activating ligands and biological functions. In addition. . .

SUMM . . . belonging to the tachykinin family of peptides, the latter being so-named because of their prompt contractile action on extravascular smooth **muscle** tissue. The tachykinins are distinguished by a conserved carboxyl-terminal sequence Phe-X-Gly-Leu-Met-NH.sub.2. In addition to SP the known mammalian tachykinins include. . .

SUMM . . . or "parent drug" refers to the biologically active entity that is released via enzymatic action of a metabolic or a **catabolic** process, or via a chemical process following administration of the prodrug. The parent compound may also be the starting material. . .

DETD . . . separated. The organic layer was washed with 100 mL of saturated aqueous sodium bicarbonate solution, 100 mL of saturated aqueous **ammonium chloride** solution, dried over magnesium sulfate and concentrated in vacuo. Crystallization from hexanes at -20.degree. C. for 72 h afforded 8.0. . .

DETD . . . for 15 min and at 25.degree. C. for 15 min. The reaction was quenched with 150 mL of saturated aqueous **ammonium chloride** solution, diluted with 300 mL of ethyl acetate, and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . and the resulting mixture was stirrred cold for 15 min. The reaction was quenched with 50 mL of saturated aqueous **ammonium chloride** solution, diluted with 50 mL of ethyl acetate, and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . resulting mixture was heated at reflux for 3 h. The reaction was cooled, quenched with 50 mL of saturated aqueous **ammonium chloride** solution, diluted with 50 mL of ethyl acetate and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . were removed in vacuo and the residue was partitioned between 20 mL of ethyl acetate and 10 mL of saturated **ammonium chloride** solution. The organic layer was separated, dried over sodium carbonate, and concentrated in vacuo. The residue was dissolved in saturated. . .

DETD . . . and the temperature was allowed to rise to 0.degree. C. The reaction was quenched with 100 mL of saturated aqueous **ammonium chloride** solution, transferred to a 1 L flask, and the ether and THF were removed in vacuo. The concentrated mixture was. . .

DETD . . . NMR was taken to confirm reaction completion (see below), then the reaction was quenched into a solution of 6% aqueous **ammonium chloride** (700 mL), maintained at 0.degree.-5.degree.. The organic phase was washed with cold water (3.times.575 mL) and brine (575mL), then was. . .

DETD The reaction was thne quenched by addition of a solution of 10% aqueous **ammonium chloride** (20 mL) over 10 min, maintaining the temperature below 10.degree. C. The layers were separated and the organic phase was. . .

DETD . . . NMR was taken to confirm reaction completion (see below), then the reaction was quenched into a solution of 6% aqueous **ammonium chloride** (700 mL), maintained at 0.degree.-5.degree. C. The layers were separated and the organic phase was washed with cold water (3.times.575. . .

DETD . . . 25.degree. C. and aged for 2.5 hours. The batch was diluted with 1:1 hexane:methyl-t-butyl ether (10 L) and 10.9% aqueous **ammonium chloride** (11 L). The phases were partitioned

and the aqueous phase was back extracted with 1:1 hexane:methyl-t-butyl ether (2.times.8 L), followed. . .

L15 ANSWER 42 OF 109 USPATFULL

AB Novel piperazine compounds promote the release of growth hormone in humans and animals. This property may be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, such as short stature in growth hormone deficient children, and to treat medical conditions which are improved by the anabolic effects of growth hormone.

Growth hormone releasing compositions containing such piperazine compounds as the active ingredient thereof are also disclosed.

AN 1998:79339 USPATFULL

TI Piperazine compounds promote release of growth hormone

IN Nargund, Ravi, East Brunswick, NJ, United States
Barakat, Khaled, Brooklyn, NY, United States
Chen, Meng Hsin, Westfield, NJ, United States
Patchett, Arthur, Westfield, NJ, United States

PA Merck & Co., Inc, Rahway, NJ, United States (U.S. corporation)

PI US 5777112 19980707 <--
WO 9534311 19951221 <--

AI US 1996-750759 19961212 (8)
WO 1995-US7001 19950609
19961212 PCT 371 date
19961212 PCT 102(e) date

RLI Continuation of Ser. No. US 1994-258644, filed on 13 Jun 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Huff, Sheela

LREP Thies, J. Eric, Rose, David L.

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 1638

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5777112 19980707 <--
WO 9534311 19951221 <--

SUMM 1. Increased rate of **protein** synthesis in all cells of the body;

SUMM . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.

SUMM . . . secretagogues may be summarized as follows: stimulating growth hormone release in elderly humans; treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids, treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating bone fracture repair,. . . syndrome, sleep disorders, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral

nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . adjunctive therapy for patients on chronic hemodialysis; treatment of immunosuppressed patients and enhancement of antibody response following vaccination; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .

SUMM . . . 100 mL of water and extracted with 3.times.50 mL of ethyl acetate. The combined organics were washed with saturated aqueous **ammonium chloride** solution, 2.times.50 mL of brine, dried over anhydrous MgSO4 and concentrated. This material was reduced to the benzylamine derivative with. . .

L15 ANSWER 43 OF 109 USPATFULL

AB A broad class of pharmaceutical agents which react directly with electron carriers or with reactive species produced by electron transport to release a pharmacologically active molecule to effect a therapeutic functional change in the organism by a receptor or nonrecepter mediated action.

AN 1998:75736 USPATFULL

TI Pro drugs for selective drug delivery

IN Mills, Randell Lee, R.D. #2, Cochranville, PA, United States 19330

PI US 5773592 19980630 <--

AI US 1995-450672 19950530 (8)

RLI Continuation of Ser. No. US 1989-446439, filed on 4 Dec 1989, now patented, Pat. No. US 5428163 which is a continuation-in-part of Ser. No. US 1986-948326, filed on 31 Dec 1986, now abandoned And a continuation-in-part of Ser. No. US 1988-175970, filed on 31 Mar 1988, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Richter, Johann

LREP Lahive & Cockfield, LLP, DeConti, Jr, Giulio A., Russett, Mark D.

CLMN Number of Claims: 26

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 3087

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5773592 19980630 <--

SUMM At least from a numerical standpoint, the **proteins** of the cell form the most important class of drug receptors. Obvious examples are the enzymes of crucial metabolic or regulatory pathways (eg., tyrosine hydroxylase; 3-hydroxy-3-methylglutaryl-CoA reductase), but of equal interest are **proteins** involved in transport processes (eg. Ca.sup.2+ -ATPase; Na.sup.+ -K.sup.+ -ATPase) or those that are **protein** kinases which activate other **proteins** as a consequence of their binding a secondary messenger such as cAMP. Specific binding properties of other cellular constituents can. . .

SUMM . . . of the Luminide agent to the blood brain barrier or cell membranes, or affinity of the Luminide agent to plasma **proteins** which results in a decreased excretion rate relative to free C, or lack of reactivity of extracellular enzymes with the. . . antineoplastic drugs, antihypertensive drugs, epinephrine blocking agents, cardiac inotropic drugs, antidepressant drugs, diuretics, antifungal agents, antibacterial drugs, anxiolytic agents, sedatives, **muscle** relaxants, anticonvulsants, agents for the treatment of ulcer disease, agents for the treatment of asthma and hypersensitivity reactions,

antithroboembolic agents,. . .
DETD . . . C functionality of a cellular and blood-brain barrier impermeant compound which enhances GABA release such as Baclofen is an anti-convulsant, **muscle** relaxant, sedative, and anxiolytic agent.
DETD . . . disruption of the degradation of purine analogue chemotherapeutic agents; the mechanism in the fourth case involves blocking the loss of **catabolic** products of adenosine triphosphate in the form of purine nucleotides and oxypurines during ischemia. Additional luminides effective in enhancing post. . .
DETD . . . D,L-2-fluoro GABA, guanidino acetic acid, 2-hydrazinopropionic acid, taurine, D,L-ornithine, or sulphanilamine potentiates the inhibitory action of GABA and is a **muscle** relaxant, anticonvulsant, sedative, and anxiolytic agent.
DETD . . . comprising a C functionality of cellular impermeant guanosine 5' cyclic monophosphate or 8-bromo guanosine 5' cyclic monophosphate which relaxes smooth **muscle** is an antihypertensive and bronchodilator agent.
DETD A cellular permeant luminide comprising a C functionality of a cellular impermeant isoquinoline-sulfonamide inhibitor of **protein** kinase C, cAMP-dependant **protein** kinase, or cGMP-dependent **protein** kinase such as N-(2-aminoethyl)-5-isoquino-linesulfonamide is an agent which blocks the secretion, contraction,
and
metabolic events regulated by these mediators of. . .
DETD A luminide. possessing more favorable pharmacokinetics or pharmacodynamics than its C moiety of an inhibitor of bacterial **protein** synthesis such as vancomycin, an aminogylcoside, erythromycin, tetracyclin, or chloramphenicol is a more efficacious antibacterial agent than the free C. . .
DETD A luminide possessing more favorable pharmacokinetics or pharmacodynamics than its C moiety which directly relaxes vascular smooth **muscle** such as hydralazine, minoxidil, or isoxsuprine is a more efficacious antihypertensive agent than the free C moiety.
DETD In addition, luminides which provide controlled extracellular release
of
biologically active substances such as drugs and **proteins** including enzymes and hormones are herein disclosed as macromolecular luminides. Luminides, each comprising a C functionality of a drug or **protein** such as insulin, erythropoietin, interleuken 2, interferon, growth hormone, atrial natriuretic factor, tissue plasminogen activator, an anti-inflammatory drug, an antihypertensive. . .
DETD . . . a pale yellow. The reaction mixture was then cooled to room temperature and cautiously treated with 45 ml of saturated **ammonium chloride** solution. This mixture was filtered and the filtrate boiled with 0.1 g of p-toluenesulphonic acid until the evolution of water. . .
DETD . . . reagent with continuous heating. After the addition is completed, the mixture is refluxed for three more hours. After cooling, sufficient **ammonium chloride** solution (saturated aqueous solution) is very carefully added in order to dissolve any free magnesium. The Grignard complex is decomposed. . .
CLM What is claimed is:
. . . . drugs, antidepressant drugs, agents for the treatment of asthma and hypersensitivity reactions, diuretics, antifungal agents, antibacterial drugs, anxiolytic agents, sedatives, **muscle** relaxants, anticonvulsants, agents for the treatment of ischemic heart disease, agents which activate the effects of secondary messengers, agents to. .

.

L15 ANSWER 44 OF 109 USPATFULL
AB The present invention is directed to certain novel compounds identified as 4-heterocycle substituted piperidines of the general structural formula: ##STR1## wherein R.sup.1, R.sup.4, R.sup.5, A, R.sup.3 and the dashed line are as defined herein. These compounds promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, such as short stature in growth hormone deficient children, and to treat medical conditions which are improved by the anabolic effects of growth hormone. Growth hormone releasing compositions containing such compounds as the active ingredient thereof are also disclosed.
AN 1998:69036 USPATFULL
TI 4-Heterocyclic peperidines promote release of growth hormone
IN Nargund, Ravi, East Brunswick, NJ, United States
Patchett, Arthur A., Westfield, NJ, United States
Yang, Lihu, Edison, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5767118 19980616 <--
AI US 1994-329357 19941026 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Covington, Raymond
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1513
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5767118 19980616 <--
SUMM . . . hormone is known to have the following basic effects on the metabolic processes of the body: (1) Increased rate of **protein** synthesis in all cells of the body; (2) Decreased rate of carbohydrate utilization in cells of the body; (3) Increased. . .
DETD . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.
DETD . . . hormone may be summarized as follows: stimulating growth hormone release in elderly humans; treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system, acceleration of wound healing; accelerating bone fracture repair;. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; treating malabsorption syndromes; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . head injury, or from infection, such as bacterial or viral infection,

especially infection with the human immunodeficiency virus; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .

DETD . . . the instant compounds are useful in the prevention or treatment of a condition selected from the group consisting of: osteoporosis; **catabolic** illness; immune deficiency, including that in individuals with a depressed T.sub.4 /T.sub.8 cell ratio; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and **protein** loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or burns,. . .

DETD . . . warm-up to room temperature and stirred for 2 h. The reaction mixture was quenched with 20 mL of saturated aqueous **ammonium chloride** solution and extracted with ethyl acetate (3.times.25 mL). The combined organics were washed with brine (50 mL), dried over MgSO.sub.4. . .

L15 ANSWER 45 OF 109 USPATFULL

AB The invention relates to compositions of erythrocytes that have been modified following hypotonic lysis and resealing by addition of 2',3'-dideoxycytidine-5'-triphosphate (ddCTP) or 3'-azido-3'-deoxythymidine-5'-triphosphate (AZT-TP). These compositions may also contain ATP. Also disclosed are methods of preparing these compositions.

AN 1998:54476 USPATFULL

TI Transformed erythrocytes, process for preparing the same, and their use in pharmaceutical compositions

IN Magnani, Mauro, Urbino, Italy
Rossi, Luigia, Urbino, Italy

PA Communaute Economique Europeene, Luxembourg, Luxembourg (non-U.S. corporation)

PI	US 5753221	19980519		<--
	WO 9222306 19921223			<--
AI	US 1993-146060	19931103 (8)		
	WO 1992-EP1291	19920609		
		19931103	PCT 371 date	
		19931103	PCT 102(e) date	
PRAI	EP 1991-401602	19910614		

DT Utility
FS Granted
EXNAM Primary Examiner: Stanton, Brian R.
LREP Bierman and Muserlian
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 814
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI	US 5753221	19980519	<--
	WO 9222306 19921223		<--

SUMM . . . of HIV replication in vitro among all the dideoxynucleoside analogs tested. ddCyd is also resistant to cytidine deaminase (a major **catabolic** enzyme for cytidine analogs), has good bioavailability, straight forward pharmacokinetic clearance by the kidney, failure to reduce normal intracellular pyrimidine. . .

SUMM . . . incubation the dishes are extensively washed with RPMI 1640 medium to remove all erythrocytes not phagocytosed, followed by a 0.9% **ammonium chloride** washing step to remove adherent erythrocytes that are not yet phagocytosed.

SUMM More particularly, the compositions of the invention are characterized in that surface **proteins** of the erythrocytes and/or transmembrane **proteins** of the erythrocytes, with said **proteins** being susceptible to be recognized by antibodies themselves susceptible to be recognized by cells of an human or animal organism,. . .

SUMM The invention also concerns compositions such as described above wherein said clustered **proteins** covalently cross-linked, are bound to said antibodies.

SUMM In order to obtain transformed erythrocytes wherein surface and/or transmembrane **proteins** are clustered such as described above, the process above described can comprise the following steps:

SUMM treating the erythrocytes with a clustering agent of surface or transmembrane **proteins**, such as ZnCl.sub.2,

SUMM covalently linking the clustered **proteins** with a cross linking agent such as bis(sulfosuccinimidyl)-suberate (BS.sup.3), with these steps being carried out, prior or after carrying out. . .

SUMM Preferably, these two steps for clustering the surface and/or transmembrane **proteins** are carried out after encapsulation.

DETD . . . mild as possible is based on an observation that ZnCl.sub.2 causes band 3 clustering (band 3 is the predominant transmembrane **protein** in mammalian erythrocytes and functions as anion transport system) and autologous IgG binding. The band 3 clusters should be make. . .

DETD The determination of erythrocytes bound autologous IgG was performed by evaluating .sup.125 I-**Protein** A binding. Briefly, following the procedure of encapsulation the loaded erythrocytes were divided into two aliquots one of which serve. . . (HEPES buffer). Washed erythrocytes (50 .mu.l) were then resuspended in 100 .mu.l of HEPES buffer containing 5.10.sup.5 c.p.m. of .sup.125 I-**Protein** A (1.1 mCi/mg **protein** A) and incubated at room temperature for 30 min. The erythrocytes were then extensively washed in HEPES buffer (four times). . .

DETD . . . incubation the dishes were extensively washed with RPMI 1640 medium to remove all erythrocytes not phagocytosed, followed by a 0.9% **ammonium chloride** washing step to remove adherent erythrocytes that were not yet phagocytosed.

DETD . . . I (LAU Bru isolate) for 8 h at a p24 concentration of 40 ng/10.sup.7 monocytes/macrophages cells. p24 is a virus **protein** described in Reitz M. S. et al., (1987). Human T-cell leukemia viruses. The molecular basis of blood diseases. Stamatoyannopoulos G.,. . .

CLM What is claimed is:
5. A transformed erythrocyte of claim 1 wherein at least one surface **protein** or transmembrane **protein** of said erythrocyte is cross-linked following treatment with a clustering agent and a **protein** cross-linking agent.

L15 ANSWER 46 OF 109 USPATFULL

AB Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life. Methods are described herein which utilize these polypeptides in treating disorders involving the LFA-1 receptor. In one of the described methods of treatment, the polypeptide

includes the amino acid sequence PKNSSMISNTP (SEQ ID NO:3) and may also
include the sequence selected from the group consisting of HQNLSDGK
(SEQ
ID NO: 1), HQNISDGK (SEQ ID NO:2), HQSLGTQ (SEQ ID NO:11) and VISSHLGQ
(SEQ ID NO:31).

AN 1998:47965 USPATFULL
TI Polypeptides with increased half-life for use in treating disorders involving the LFA-1 receptor
IN Presta, Leonard G., San Francsico, CA, United States
Snedecor, Bradley R., Portola Valley, CA, United States
PA Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 5747035 19980505 <--
AI US 1995-422091 19950414 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Gambel, Phillip
LREP Dreger, Walter H.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 3305
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5747035 19980505 <--

SUMM . . . (pFc') fragment of human IgG also produced by trypsin digestion of the Fc fragment was rapidly eliminated, indicating that the **catabolic** site of IgG is located in the CH2 domain. Ellerson et al., J. Immunol., 116: 510 (1976); Yasmeen et al.,. . .

SUMM The **catabolic** rates of IgG variants that do not bind the high-affinity Fc receptor FcRI or C1q are indistinguishable from the rate of clearance of the parent wild-type antibody, indicating that the **catabolic** site is distinct from the sites involved in FcRI or C1q binding. Wawrzynczak et al., Molec. Immunol., 29: 221 (1992).. . .

SUMM Staphylococcal **protein** A-IgG complexes were found to clear more rapidly from the serum than uncomplexed IgG molecules. Dima et al., Eur. J.. . . on the pharmacokinetics of the Fc-hinge fragment. The authors showed that the site of the IgG1 molecule that controls the **catabolic** rate (the "**catabolic** site") is located at the CH2-CH3 domain interface and overlaps with the Staphylococcal **protein** A binding site. See also WO 93/22332 published Nov. 11, 1993. The concentration catabolism phenomenon is also studied in Zuckier. . .

SUMM WO 94/04689 discloses a **protein** with a cytotoxic domain, a ligand-binding domain and a peptide linking these two domains comprising an IgG constant region domain having the property of increasing the half-life of the **protein** in mammalian serum.

SUMM A stereo drawing of a human Fc fragment and its complex with fragment B of **Protein** A from Staphylococcus aureus is provided by Deisenhofer, Biochemistry, 20: 2364 (1981).

DETD . . . as is well known to those skilled in the art of antibody technology. Examples of such polypeptides are peptides and **proteins**, whether from eukaryotic sources such as, e.g., yeast, avians, plants, insects, or mammals, or from bacterial sources such as, e.g.,. . .

DETD . . . hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting

factors such as **Protein** C; atrial naturietic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA);. . . a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial **protein**, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; **protein** A or D; rheumatoid factors; a neurotrophic factor such as brain-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3,. . . TGF-.beta.2, TGF-.beta.3, TGF-.beta.4, or TGF-.beta.5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding **proteins**; CD **proteins** such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic **protein** (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs),. . . IL-1 to IL-10; an anti-HER-2 antibody without a native Fc region of an IgG; superoxide dismutase; T-cell receptors; surface membrane **proteins**; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport **proteins**; homing receptors; addressins; regulatory **proteins**; antibodies without a native Fc region of an IgG; and fragments of any of the above-listed polypeptides.

DETD Libraries are screened with probes designed to identify the gene of interest or the **protein** encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to. . .

DETD . . . In some preferred embodiments, the nucleic acid sequence includes the polypeptide of interest's signal sequence. Nucleic acid having all the **protein** coding sequence is obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for. . .

DETD . . . insertions include insertions to the internal portion of the polypeptide of interest, as well as N- or C-terminal fusions with **proteins** or peptides containing the desired epitope that will result, upon fusion, in an increased half-life.

DETD . . . bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for **protein** production, generally an expression vector of the type typically employed for transformation of an appropriate host.

DETD Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a **protein** necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode **proteins** that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or. . .

DETD . . . drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a **protein** conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et. . .

DETD . . . that encodes the polypeptide variant. Amplification is the process by which genes in greater demand for the production of a

protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the. . .

DETD . . . cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the polypeptide variant, wild-type DHFR **protein**, and another selectable marker such as aminoglycoside 3-phosphotransferase (APH) can be selected by cell growth in medium containing a selection. . .

DETD . . . amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding **proteins** endogenous to the host, with examples of such hosts including E. coli W3110 strain 1A2, which has the complete genotype. . .

DETD . . . Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-**protein** duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound. . .

DETD . . . step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration; optionally, the **protein** may be concentrated with a commercially available **protein** concentration filter, followed by separating the polypeptide variant from other impurities by one or more steps selected from immunoaffinity chromatography,. . . groups), chromatography on Blue-SEPHAROSE, CM BLUE-SEPHAROSE, MONO-Q, MONO-S, LENTIL LECTIN-SEPHAROSE, WGA-SEPHAROSE, CON A-SEPHAROSE, ETHER TOYOPEARL, BUTYL TOYOPEARL, PHENYL TOYOPEARL, or **protein** A SEPHAORSE, SDS-PAGE chromatography, silica chromatography, chromatofocusing, reverse phase HPLC (e.g., silica gel with appended aliphatic groups), gel filtration using,. . .

DETD . . . another embodiment, supernatants from systems which secrete recombinant polypeptide variant into culture medium are first concentrated using a commercially available **protein** concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate may be applied to a suitable purification matrix. For example, a suitable affinity matrix may comprise a ligand for the **protein** , a lectin or antibody molecule bound to a suitable support. Alternatively, an anion-exchange resin may be employed, for example, a. . . matrix or substrate having pendant DEAE groups. Suitable matrices include acrylamide, agarose, dextran, cellulose, or other types commonly employed in **protein** purification. Alternatively, a cation-exchange step may be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups.. . .

DETD Mammalian polypeptide variant synthesized in recombinant culture is characterized by the presence of non-human cell components, including **proteins**, in amounts and of a character which depend on the purification steps taken to recover the polypeptide variant from culture.. . .

DETD . . . O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using .sup.125 I or .sup.131 I to prepare labeled **proteins** for use in radioimmunoassay, the chloramine T method described above being suitable.

DETD . . . of seryl or threonyl residues, methylation of the .alpha.-amino

groups of lysine, arginine, and histidine side chains (T. E. Creighton, **Proteins**: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and. . .

DETD . . . the compound tunicamycin as described by Duskin et al., J. Biol. Chem., 257: 3105 (1982). Tunicamycin blocks the formation of **protein**-N-glycoside linkages.

DETD . . . be fused to a second polypeptide and the antibody or fusion thereof may be used to isolate and purify the **protein** to which it binds from a source such as a CD11 or CD18 antigen. In another embodiment, the invention provides. . .

DETD . . . as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; **proteins**, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine. . .

DETD . . . (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a **protein** that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin. . .

DETD . . . the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different **protein** and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as **protein** fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

DETD . . . as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the **protein** used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable. . .

DETD . . . are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, **protein** A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DETD . . . E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin **protein**, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of. . .

DETD Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic **protein** chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a. . .

DETD . . . using leucine zippers. Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun **proteins** were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the. . .

DETD . . . tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or variant antibody, and **proteins** that bind to the analyte are denominated binding partners, whether they be antibodies, cell-surface receptors, or antigens.

DETD . . . flanking the F1 origin were removed from pB0475 and DNA coding for anti-CD18 Fab H52, version OZ (Eigenbrot et al., **Proteins**, 18: 49-62 [1994]) was substituted for DNA coding for human growth hormone using the EcoRV and Sphl sites. Hence, pH52. . .

DETD . . . 0.3 g yeast extract certified (Difco.TM. 0127-01-7), 0.19 g MgSO.sub.4 anhydrous or 0.394 g MgSO.sub.4.7H.sub.2 O (Sigma.TM. M2773), 1.07 g **ammonium chloride** (Sigma.TM. A9434), 0.075 g KCl (Sigma.TM. P5405), 4.09 g NaCl (Sigma.TM. S3014), 120.0 mL of 1M triethanolamine pH 7.4, qs. . .

DETD The supernatant was then passed over a **Protein** G-Sepharose.TM. Fast Flow (Pharmacia) column [0.5 mL bed volume] previously equilibrated by passing 10 mL TE buffer through the column.. . . added to concentrated eluant, and the resulting mixture was re-concentrated to 0.5 mL. SDS-PAGE gels were run to ascertain that **protein** had been produced.

DETD . . . out on a reverse-phase PLRP-S.TM. 4.6.times.50 mm column, 8-mm particle size (Polymer Laboratories, Shropshire, UK), maintained at 50.degree. C. The **proteins** were eluted using an increasing linear gradient from 31% B to 41% B. Buffer A contained 0.1% trifluoroacetic acid in. . . and Buffer B contained 0.1% trifluoroacetic acid in HPLC-grade acetonitrile. The flow rate was maintained at 2 mL/min, and the **protein** profile was monitored at 214 nm.

DETD . . . carried out on a Bakerbond carboxy-sulfon (CSX).TM. 50.times.4.6 mm column (J. T. Baker Phillipsburg, N.J.), maintained at 55.degree. C. The **proteins** were eluted using an increasing linear gradient from pH 6.0 to pH 8.0 at a flow rate of 2 mL/min. . .

DETD SDS-PAGE analysis was carried out on precast Novex.TM. gels (Novex, San Diego, Calif.). The **proteins** were stained using the Morrissey silver stain method. Morrissey, Anal. Biochem., 117: 307-310 (1981).

DETD . . . test (Associates of Cape Cod Inc., Woods Hole, Mass.). Samples containing less than 2 endotoxin units (Eu) per mg of **protein** were used in the pharmacokinetic studies.

DETD . . . 3.5. To this solution, pepsin (1 mg/mL) dissolved in 100 mM sodium citrate buffer, pH 3.5, was added at a pepsin-to-**protein** ratio of 1:12. After 4 hours at room temperature, the mixture's pH was raised to pH 6.4 with 10% NaOH.

DETD . . . described above for the Fab antibody fragment variants. After endotoxin determinations, samples containing less than 2 Eu per mg of **protein** were used in the pharmacokinetic studies set forth below.

L15 ANSWER 47 OF 109 USPATFULL

AB In this disclosure, there are provided materials which completely degrade in the environment far more rapidly than pure synthetic plastics but which possesses the desirable properties of a thermoplastic: strength, impact resistance, stability to aqueous acid or base, and deformation at higher temperatures. There is provided a method for using the degradable plastic materials in preparing strong, moldable solids. There is further provided a method of making and applications for macromolecular, surface active agents that change the wetting behavior of lignin-containing materials. These surface active agents are used to provide a method of making and applications for synthetic polymers coupled to pieces of a vascular plant using macromolecular surface active agents.

AN 1998:42440 USPATFULL

TI Biodegradable plastics and composites from wood
IN Meister, John J., 31675 Westlady Rd., Beverly Hills, MI, United States 48025-3744
Chen, Meng-Jiu, 901 St. Louis, Apt. #25, Ferndale, MI, United States 48220
PI US 5741875 19980421 <--
AI US 1995-400891 19950308 (8)
RLI Continuation-in-part of Ser. No. US 1993-80006, filed on 21 Jun 1993, now patented, Pat. No. US 5424382 which is a continuation-in-part of Ser. No. US 1991-789360, filed on 8 Nov 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Truong, Duc
LREP Barnes, Kisselle, Raisch, Choate, Whittemore & Hulbert P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 2237
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5741875 19980421 <--
DETD . . . cellulose, hemicellulose, and lignin, possibly contaminated with the inert "mineral" portion of the plant: starch, lipid, silica bodies, silica stegmata, **protein** bodies, and mucilage.
DETD TABLE 4

Some Halides Useful in Polymerization of Lignin-Containing Materials.

Calcium Chloride	Magnesium Chloride	Sodium Chloride
Potassium Chloride	Lithium Chloride	**Ammonium Chloride**
Calcium Bromide	Magnesium Bromide	Sodium Bromide
Potassium Bromide	Lithium Bromide	Ammonium Bromide
Calcium Fluoride	Magnesium Fluoride	Sodium Fluoride
Potassium Fluoride	Lithium Fluoride	Ammonium Fluoride

DETD . . . with brown rot fungus Gloeophyllum trabeum. Three of these fungi are white-rot species that attack and degrade woody materials by **catabolic** activity while the fourth fungus is a brown-rot that acts as a negative control since it attacks woody materials by. . .

L15 ANSWER 48 OF 109 USPATFULL
AB Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.
AN 1998:39666 USPATFULL
TI Altered polypeptides with increased half-life

IN   Presta, Leonard G., San Francisco, CA, United States
        Snedecor, Bradley R., Portola Valley, CA, United States
PA   Genentech Inc., San Francisco, CA, United States (U.S. corporation)
PI   US 5739277   19980414   <--
AI   US 1995-422101   19950414 (8)
DT   Utility
FS   Granted
EXNAM   Primary Examiner: Feisee, Lila; Assistant Examiner: Johnson, Nancy A.
LREP   Hasak, Janet E.
CLMN   Number of Claims: 3
ECL   Exemplary Claim: 1,2,3
DRWN   4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 3251
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5739277   19980414   <--

SUMM . . . (pFc') fragment of human IgG also produced by trypsin digestion of the Fc fragment was rapidly eliminated, indicating that the **catabolic** site of IgG is located in the CH2 domain. Ellerson et al, J. Immunol., 116: 510 (1976); Yasmeen et al.,. . .

SUMM The **catabolic** rates of IgG variants that do not bind the high-affinity Fc receptor FcRI or C1q are indistinguishable from the rate of clearance of the parent wild-type antibody, indicating that the **catabolic** site is distinct from the sites involved in FcRI or C1q binding. Wawrzynczak et al., Molec. Immunol., 29: 221 (1992).. . .

SUMM Staphylococcal **protein** A-IgG complexes were found to clear more rapidly from the serum than uncomplexed IgG molecules. Dima et al., Eur. J.. . . on the pharmacokinetics of the Fc-hinge fragment. The authors showed that the site of the IgG1 molecule that controls the **catabolic** rate (the "**catabolic** site") is located at the CH2-CH3 domain interface and overlaps with the Staphylococcal **protein** A binding site. See also WO 93/22332 published Nov. 11, 1993. The concentration catabolism phenomenon is also studied in Zuckier. . .

SUMM WO 94/04689 discloses a **protein** with a cytotoxic domain, a ligand-binding domain and a peptide linking these two domains comprising an IgG constant region domain having the property of increasing the half-life of the **protein** in mammalian serum.

SUMM A stereo drawing of a human Fc fragment and its complex with fragment B of **Protein** A from Staphylococcus aureus is provided by Deisenhofer, Biochemistry, 20: 2364 (1981).

DETD . . . as is well known to those skilled in the art of antibody technology. Examples of such polypeptides are peptides and **proteins**, whether from eukaryotic sources such as, e.g., yeast, avians, plants, insects, or mammals, or from bacterial sources such as, e.g.,. . .

DETD . . . hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as **Protein** C; atrial naturietic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA);. . . a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial **protein**, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for

hormones or growth factors; integrin; **protein** A or D; rheumatoid factors; a neurotrophic factor such as brain-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3,. . . TGF-.beta.2, TGF-.beta.3, TGF-.beta.4, or TGF-.beta.5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding **proteins**; CD **proteins** such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic **protein** (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs) e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs),. . . IL-1 to IL-10; and anti-HER-2 antibody without a native Fc region of an IgG; superoxide dismutase; T-cell receptors; surface membrane **proteins**; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport **proteins**; homing receptors; addressins; regulatory **proteins**; antibodies without a native Fc region of an IgG; and fragments of any of the above-listed polypeptides.

DETD Libraries are screened with probes designed to identify the gene of interest or the **protein** encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to. . .

DETD . . . In some preferred embodiments, the nucleic acid sequence includes the polypeptide of interest's signal sequence. Nucleic acid having all the **protein** coding sequence is obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for. . .

DETD . . . insertions include insertions to the internal portion of the polypeptide of interest, as well as N- or C-terminal fusions with **proteins** or peptides containing the desired epitope that will result, upon fusion, in an increased half-life.

DETD . . . bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for **protein** production, generally an expression vector of the type typically employed for transformation of an appropriate host.

DETD Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a **protein** necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode **proteins** that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or. . .

DETD . . . drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a **protein** conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et. . .

DETD . . . that encodes the polypeptide variant. Amplification is the process by which genes in greater demand for the production of a **protein** critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the. . .

DETD . . . cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the polypeptide variant, wild-type DHFR **protein**, and another selectable marker such as aminoglycoside 3-phosphotransferase (APH) can be selected by cell growth in medium containing a selection. . .

DETD . . . amounts of proteolytic enzymes. For example, strain W3110 may

be modified to effect a genetic mutation in the genes encoding **proteins** endogenous to the host, with examples of such hosts including E. coli W3110 strain 1A2, which has the complete genotype. . .

DETD . . . Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-**protein** duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound. . .

DETD . . . step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration; optionally, the **protein** may be concentrated with a commercially available **protein** concentration filter, followed by separating the polypeptide variant from other impurities by one or more steps selected from immunoaffinity chromatography,. . . groups), chromatography on Blue-Sepharose, CM Blue-Sepharose, MONO-Q, MONO-S, lentil lectin-Sepharose, WGA-Sepharose, Con A-Sepharose, Ether Toyopearl, Butyl Toyopearl, Phenyl Toyopearl, or **protein** A Sepharose, SDS-PAGE chromatography, silica chromatography, chromatofocusing, reverse phase HPLC (e.g., silica gel with appended aliphatic groups), gel filtration using,. . .

DETD . . . another embodiment, supernatants from systems which secrete recombinant polypeptide variant into culture medium are first concentrated using a commercially available **protein** concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate may be applied to a suitable purification matrix. For example, a suitable affinity matrix may comprise a ligand for the **protein** , a lectin or antibody molecule bound to a suitable support. Alternatively, an anion-exchange resin may be employed, for example, a. . . matrix or substrate having pendant DEAE groups. Suitable matrices include acrylamide, agarose, dextran, cellulose, or other types commonly employed in **protein** purification. Alternatively, a cation-exchange step may be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups.. . .

DETD Mammalian polypeptide variant synthesized in recombinant culture is characterized by the presence of non-human cell components, including **proteins**, in amounts and of a character which depend on the purification steps taken to recover the polypeptide variant from culture.. . .

DETD . . . O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using .sup.125 I or .sup.131 I to prepare labeled **proteins** for use in radioimmunoassay, the chloramine T method described above being suitable.

DETD . . . of seryl or threonyl residues, methylation of the .alpha.-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, **Proteins**: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and. . .

DETD . . . the compound tunicamycin as described by Duskin et al., J. Biol. Chem., 257: 3105 (1982). Tunicamycin blocks the formation of **protein**-N-glycoside linkages.

DETD . . . be fused to a second polypeptide and the antibody or fusion

thereof may be used to isolate and purify the`**protein** to which
it binds from a source such as a CD11 or CD18 antigen. In another
embodiment, the invention provides. . .

DETD . . . as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; **proteins**, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine. . .

DETD . . . (ip) injections of the relevant antigen and an adjuvant. It may
be useful to conjugate the relevant antigen to a **protein** that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin. . .

DETD . . . the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different **protein** and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as **protein** fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

DETD . . . as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the **protein** used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable. . .

DETD . . . are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as,
for example, **protein** A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DETD . . . E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin **protein**, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of. . .

DETD Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic **protein** chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a. . .

DETD . . . using leucine zippers. Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun **proteins** were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the. . .

DETD . . . tested is referred to herein as an analyte, irrespective of its
status otherwise as an antigen or variant antibody, and **proteins** that bind to the analyte are denominated binding partners, whether they be antibodies, cell-surface receptors, or antigens.

DETD . . . flanking the F1 origin were removed from pB0475 and DNA coding for anti-CD18 Fab H52, version OZ (Eigenbrot et al., **Proteins**, 18: 49-62 [1994]) was substituted for DNA coding for human growth hormone using the EcoRV and Sphl sites. Hence, pH52. . .

DETD . . . 0.3 g yeast extract certified (Difco.TM. 0127-01-7), 0.19 g MgSO.sub.4 anhydrous or 0.394 g MgSO.sub.4.7H.sub.2 O (Sigma.TM. M2773),
1.07 g **ammonium chloride** (Sigma.TM. A9434), 0.075 g

KCl (Sigma.TM. P5405), 4.09 g NaCl (Sigma.TM. S3014), 120.0 mL of 1M triethanolamine pH 7.4, qs. . . .

DETD The supernatant was then passed over a **Protein** G-Sepharose.TM. Fast Flow (Pharmacia) column [0.5 mL bed volume] previously equilibrated by passing 10 mL TE buffer through the column.. . . . added to concentrated eluant, and the resulting mixture was re-concentrated to 0.5 mL. SDS-PAGE gels were run to ascertain that **protein** had been produced.

DETD . . . out on a reverse-phase PLRP-S.TM. 4.6.times.50 mm column, 8-mm particle size (Polymer Laboratories, Shropshire, UK), maintained at 50.degree. C. The **proteins** were eluted using an increasing linear gradient from 31% B to 41% B. Buffer A contained 0.1% trifluoroacetic acid in. . . . and Buffer B contained 0.1% trifluoroacetic acid in HPLC-grade acetonitrile. The flow rate was maintained at 2 mL/min, and the **protein** profile was monitored at 214 nm.

DETD . . . . carried out on a Bakerbond carboxy-sulfon (CSX).TM. 50.times.4.6 mm column (J. T. Baker Phillipsburg, N.J.), maintained at 55.degree. C. The **proteins** were eluted using an increasing linear gradient from pH 6.0 to pH 8.0 at a flow rate of 2 mL/min. . . .

DETD SDS-PAGE analysis was carried out on precast Novex.TM. gels (Novex, San Diego, Calif.). The **proteins** were stained using the Morrissey silver stain method. Morrissey, Anal. Biochem., 117: 307-310 (1981).

DETD . . . . test (Associates of Cape Cod Inc., Woods Hole, Mass.). Samples containing less than 2 endotoxin units (Eu) per mg of **protein** were used in the pharmacokinetic studies.

DETD . . . . 3.5. To this solution, pepsin (1 mg/mL) dissolved in 100 mM sodium citrate buffer, pH 3.5, was added at a pepsin-to-**protein** ratio of 1:12. After 4 hours at room temperature, the mixture's pH was raised to pH 6.4 with 10% NaOH.

DETD . . . . described above for the Fab antibody fragment variants. After endotoxin determinations, samples containing less than 2 Eu per mg of **protein** were used in the pharmacokinetic studies set forth below.

L15 ANSWER 49 OF 109 USPATFULL

AB The present invention is directed to certain novel compounds identified as bridged piperidines of the general structural formula: ##STR1## wherein R.sup.1, R.sup.1a, R.sup.2a, R.sup.3, R.sup.3a,R.sup.4, R.sup.5, A, X, and Y are as defined herein. These compounds promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, such as short stature in growth hormone deficient children, and to treat medical conditions which are improved by the anabolic effects of growth hormone. Growth hormone releasing compositions containing such compounds as the active ingredient thereof are also disclosed.

AN 1998:31024 USPATFULL

TI Bridged piperidines promote release of growth hormone

IN Lu, Zhijian, Scotch Plains, NJ, United States
Patchett, Arthur A., Westfield, NJ, United States
Tata, James R., Westfield, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5731317 19980324 <--

AI US 1995-401849 19950310 (8)

DT   Utility
FS   Granted
EXNAM   Primary Examiner: Marquis, Melvyn I.; Assistant Examiner: Harrison, Robert H.
LREP   Thies, J. Eric, Rose, David L.
CLMN   Number of Claims: 5
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 1591
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5731317   19980324   <--

SUMM   . . . hormone is known to have the following basic effects on the metabolic processes of the body: (1) Increased rate of **protein** synthesis in all cells of the body; (2) Decreased rate of carbohydrate utilization in cells of the body; (3) Increased. . .

SUMM   . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.

SUMM   . . . uses as growth hormone itself. These varied uses may be summarized as follows: treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system, acceleration of wound healing; accelerating bone fracture repair;. . .

syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; treating malabsorption syndromes; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . virus; treatment of syndromes manifested by non-restorative sleep and musculoskeletal pain, including fibromyalgia syndrome or chronic fatigue syndrome; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .

SUMM   . . . the instant compounds are useful in the prevention or treatment of a condition selected from the group consisting of: osteoporosis; **catabolic** illness; immune deficiency, including that in individuals with a depressed T.sub.4 /T.sub.8 cell ratio; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and **protein** loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or bums,. . .

DETD   . . . added slowly. The solution was then gradually warmed up to room temperature and stirred for another 3 hr. Saturated aqueous **ammonium chloride** was added and the THF was removed. The aqueous was extracted with EtOAc (3.times.100 ml). The combined EtOAc layers were. . .

DETD   . . . 26.67 mmol) in THF (50 ml) was added. The whole was stirred at -78.degree. C. for 2 hr. Saturated aqueous **ammonium**

chloride (50 ml) was added and the THF was removed. The aqueous
was then extracted with EtOAc (3.times.60 ml). The combined. . .

L15 ANSWER 50 OF 109 USPATFULL

AB Therapeutic agents and methods for treating and diagnosing acute or chronic leukemia are provided. Such agents comprises monoclonal antibody M195, or a chimeric antibody containing the hypervariable region of M195, conjugated to a cytotoxic agent, e.g. a radioisotope.

AN 1998:30697 USPATFULL

TI Therapeutic use of hypervariable region of monoclonal antibody M195 and constructs thereof

IN Scheinberg, David A., New York, NY, United States

PA Sloan-Kettering Institute for Cancer Research, New York, NY, United States (U.S. corporation)

PI US 5730982 19980324 <--

AI US 1995-383615 19950202 (8)

RLI Continuation of Ser. No. US 1993-56957, filed on 3 May 1993, now abandoned which is a continuation of Ser. No. US 1989-450918, filed on 14 Dec 1989, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Johnson, Nancy A.

LREP White, John P.

CLMN Number of Claims: 21

ECL Exemplary Claim: 1

DRWN 25 Drawing Figure(s); 17 Drawing Page(s)

LN.CNT 2528

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5730982 19980324 <--

SUMM . . . target antigen is not expressed on any other hematopoietic or non-hematopoietic tissue. Antibodies to a related antigen on the same **protein** (CD33), My9 and L4F3, are currently being used to purge bone marrow of ANLL before autologous transfusion (Bernstein I D,. . .

SUMM . . . myeloblasts, and leukemic blasts from most patients with ANLL and acute lymphoid leukemias (19-21 Ref.b). mAb NHL-30.5 identifies a 180-kDa **protein** found on a similar distribution of cells (22, 23 Ref.b). My9 and L4F3 antibodies identify a 67-kDa glycoprotein (CD33) (24-27. . .

DETD . . . an antigen restricted to early myeloid cells, monocytic cells, and ANLL. The antigen appears to be carried on the CD33 **protein** . The antigen is not detectable on any other adult tissues and thus may be useful in the study of myelomonocytic. . .

DETD . . . hybridoma cultures were screened against a panel of leukemia cell lines and the original ANLL leukemia cells using Staphylococcus aureus **protein** A (PA) erythrocyte rosetting (see below). The repeatedly sub-cloned M195 hybridoma was expanded in the peritoneal cavity of doubly pristane-primed. . .

DETD . . . 37.degree. C. Polymorphonuclear leukocytes were purified from contaminative red blood cells after dextran sedimentation at 1.times.g for 60 min by **ammonium chloride** lysis in Tris buffer at pH 7.2. Platelets were separated from the Ficoll-Paque interface cells by differential centrifugation. E-rosette-positive and. . . and neuraminidase- (Calbiochem) treated sheep red blood cells (GIBCO), followed by Ficoll-Paque gradient centrifugation and lysis of red cells with **ammonium chloride**.

DETD . . . using chloramine-T to start and sodium metabisulfite to stop

the reaction. Specific activity was between 2 and 10 .mu.Ci/.mu.g of **protein**. Immunoreactivity was between 40 and 60% as determined by serial binding to an excess of live HL60 cells. Radioimmunoassays were. . .

DETD . . . and sedimenting the pepsin beads at 15,000.times.g for 1 min. Undigested immunoglobulins and Fc fragments were removed by reaction with **Protein** A Sepharose (Pharmacia). Purity of fragments was determined by SDS-polyacrylamide gel fractionation followed by Coomassie blue staining.

DETD . . . 00 P

PHA blasts 0

(n = 5)

P = positive; W = weakly positive; 0 = negative

*As determined by direct **Protein** A and mixed hemeadherence rosseting and absorption assays as described in the text.

DETD . . . min eliminated all binding activity in radioimmunoassays and rosetting assays. This suggested that the antigen epitope is carried on a **protein**. However, treatment with trypsin, protease, and neuraminidase had no effects on binding of mAB M195 to HL60 cells. These experiments,. . . the target, other data shown in the accompanying paper (30 Ref.b) indicated that the antigen was carried on the CD33 **protein**.

DETD . . . and binding to CD33 transfectants (discussed in Experiment 2 and 30 Ref.b) demonstrated that M195 was carried on the CD33 **protein**. However, cotyping on fresh leukemias showed that the antigen detected by mAb M195 was not identical to the other CD33. . .

DETD Radioimmunoassays. M195 IgG2a was purified by **protein** A affinity chromatography, radiolabeled with iodine-125, and used in direct radioimmunoassays on live leukemia and bone marrow cells as described before (16 Ref.a). M195 was labeled to 2-10 .mu.Ci/.mu.g **protein**. Specific binding was determined by subtracting the amount of M195 IgG2a bound in the presence of an excess of unlabeled. . .

DETD . . . previous paper suggested that the distribution of M195 appeared similar to that described for CD33-reactive antibodies MY9 and L4F3. The **protein** target of M195 has thus far eluded detection (16 Ref.a). A comparison of M195 reactivity to other well characterized myeloid. . .

DETD The close coexpression of M195 and MY9 suggested that M195 might bind to the CD33 **protein** target [p67] (20 Ref.a). Efforts to identify the M195 target have been unsuccessful (16 Ref.a). Blocking experiments shown here demonstrated probable identity of the M195 target with the CD33 **protein**. Moreover, binding of M195 and CD33 DNA transfectants was shown. Despite these data, since flow cytometry data showed nonidentical concordance. . .

DETD . . . KD cell-surface glycoprotein found on most myeloid leukemia cells (24,25 Ref.c). The antibody was purified from mouse ascites fluid using **Protein** A affinity chromatography. M195 F(ab').sub.2 was prepared by pepsin digestion of the intact immunoglobulin; OKB7 Fab was prepared by papain. . .

DETD . . . of antibody from the surface by indirect methods (indirect radioimmunoassay and complement fixation) as well. The percent

radioiodine bound to **protein** in the supernatant was estimated
by TCA precipitation of the supernatant; it was never less than 95%.
Similarly, **protein**-bound radioindium was estimated in aliquots
of selected supernatants by thin layer chromatography and was always
greater than 90%.

DETD . . . percent internalization was considerably greater than observed with .sup.125 I-labeled antibody, perhaps due to transchelation of .sup.111 In to cellular **protein**.

DETD . . . target antigen is not expressed on any other hematopoietic or non-hematopoietic tissue. Antibodies to a related antigen on the same **protein** (CD33), My9 and L4F3, are currently being used to purge bone marrow of ANLL before autologous transfusion (Bernstein I D,. . .

DETD . . . for example, a new gene or part of a gene required for enzyme or hemoglobin function or another required structural **protein**, is attached to M195 by a retroviral vector. Retroviral vectors usually require receptors for entry into target cells. M195 will substitute for the usual envelope **protein** involved in this entry and will thus confer specificity for the appropriate cells.

DETD . . . the outside of the vector by chemical or genetic means: the M195 could be directly crosslinked to the viral envelope **proteins**; the M195 could be bound to another antibody or fragment which is directed against the viral envelope **proteins** ; **protein** A can be inserted genetically into the envelope to bind M195; or M195 can be inserted genetically into the envelope.. . .

DETD 14. Scheinberg D. A., Strand M. Kinetic and **Catabolic** Considerations of Monoclonal Antibody Targeting in Erythroleukemic Mice. Cancer Res. 43:265-272 (1983).

L15 ANSWER 51 OF 109 USPATFULL

AB There are disclosed certain compounds identified as substituted dipeptide analogs which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to increase the stature of those afflicted with a lack of a normal secretion of natural growth hormone. Growth promoting compositions containing such substituted dipeptide analogs as the active ingredient thereof are also disclosed.

AN 1998:25372 USPATFULL

TI Biphenyl substituted dipeptide analogs promote release of growth hormone

IN Lin, Peter, Iselin, NJ, United States
Schoen, William R., Edison, NJ, United States
Pisano, Judith M., Cliffside Park, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5726319 19980310 <--

AI US 1995-510026 19950801 (8)

RLI Continuation of Ser. No. US 1993-175809, filed on 30 Dec 1993, now abandoned which is a continuation-in-part of Ser. No. US 1992-973142, filed on 6 Nov 1992, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Tsang, Cecilia J.; Assistant Examiner: Celsa, Bennett

LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 4903
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5726319 19980310 <--
SUMM 1. Increased rate of **protein** synthesis in all cells of the body;
SUMM . . . These varied uses of growth hormone may be summarized as follows: stimulating growth hormone release in elderly humans; Prevention of **catabolic** side effects of glucocorticoids, treatment of osteoporosis, stimulation of the immune system, treatment of retardation, acceleration of wound healing, accelerating. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; Attenuation of **protein catabolic** response after a major operation; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS. Treatment of hyperinsulinemia including nesidioblastosis;
Adjuvant
treatment for ovulation induction; To stimulate thymic development and prevent the age-related decline of thymic function; Treatment of immunosuppressed patients; Improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; Stimulation of osteoblasts, bone remodelling, and. . .
DETD . . . at room temperature overnight then diluted with 1 L of methylene chloride and washed with 500 mL of saturated aqueous **ammonium chloride**, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over magnesium. . .

L15 ANSWER 52 OF 109 USPATFULL
AB There are disclosed certain novel compounds identified as benzo-fused lactams which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more
efficient,
and in humans, to increase the stature of those afflicted with a lack
of
a normal secretion of natural growth hormone. Growth promoting compositions containing such benzo-fused lactams as the active ingredient thereof are also disclosed.
AN 1998:25360 USPATFULL
TI Benzo-fused lactams promote release of growth hormone
IN Schoen, William R., Edison, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5726307 19980310 <--
AI US 1994-356935 19941215 (8)
RLI Division of Ser. No. US 1992-961008, filed on 14 Oct 1992, now
patented,
Pat. No. US 5374721, issued on 20 Dec 1994
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 6
ECL Exemplary Claim: 1

DRWN   No Drawings
LN.CNT 2191
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5726307   19980310   <--
SUMM   1. Increased rate of **protein** synthesis in all cells of the body;
SUMM   . . . These varied uses of growth hormone may be summarized as follows: stimulating growth hormone release in elderly humans; Prevention of **catabolic** side effects of glucocorticoids, treatment of osteoporosis, stimulation of the immune system, treatment of retardation, acceleration of wound healing, accelerating. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; Attenuation of **protein catabolic** response after a major operation; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS. Treatment of hyperinsulinemia including nesidioblastosis; Adjuvant treatment for ovulation induction; To stimulate thymic development and prevent the age-related decline of thymic function; Treatment of immunosuppressed patients; Improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; Stimulation of osteoblasts, bone remodelling, and. . .
DETD   . . . at room temperature overnight then diluted with 1 L of methylene chloride and washed with 500 mL of saturated aqueous **ammonium chloride**, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over magnesium. . .

L15 ANSWER 53 OF 109 USPATFULL
AB   The present invention is directed to certain novel compounds identified as substituted piperidines, pyrrolidines and hexahydro-1H-azepines of the general structural formula: ##STR1## wherein R.sub.1, R.sub.4, R.sub.5, A, X, Y and n are as defined herein. These compounds promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, such as short stature in growth hormone deficient children, and to treat medical conditions which are improved by growth hormone. Growth hormone releasing compositions containing such compounds as the active ingredient thereof are also disclosed.
AN   1998:19718 USPATFULL
TI   Piperidine, pyrrolidine and hexahydro-1H-azepines promote release of growth hormone
IN   Chen, Meng H., Westfield, NJ, United States
       Nargund, Ravi, East Brunswick, NJ, United States
       Patchett, Arthur A., Westfield, NJ, United States
       Yang, Lihu, Edison, NJ, United States
PA   Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI   US 5721251   19980224   <--
AI   US 1996-600912   19960213 (8)
RLI   Continuation-in-part of Ser. No. US 1994-323998, filed on 17 Oct 1994, now patented, Pat. No. US 5492920 which is a continuation-in-part of Ser. No. US 1993-165149, filed on 10 Dec 1993, now abandoned
DT   Utility
FS   Granted
EXNAM Primary Examiner: Chang, Ceila

LREP   Thies, J. Eric, Rose, David L.
CLMN   Number of Claims: 3
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 4349
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5721251   19980224   <--

SUMM   . . . hormone is known to have the following basic effects on the metabolic processes of the body: (1) Increased rate of **protein** synthesis in all cells of the body; (2) Decreased rate of carbohydrate utilization in cells of the body; (3) Increased. . .

SUMM   . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.

SUMM   . . . uses may be summarized as follows: stimulating growth hormone release in elderly humans; treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system, acceleration of wound healing; accelerating bone fracture repair;. . .

vascular resistance, diminishing or preventing loss of body weight and enhancing recovery following congestive heart failure; increasing appetite; attenuation of **protein catabolic** response after a major operation; treating malabsorption syndromes; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . virus; treatment of syndromes manifested by non-restorative sleep and musculoskeletal pain, including fibromyalgia syndrome or chronic fatigue syndrome; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .

SUMM   . . . the instant compounds are useful in the prevention or treatment of a condition selected from the group consisting of: osteoporosis; **catabolic** illness; immune deficiency, including that in individuals with a depressed T.sub.4 /T.sub.8 cell ratio; bone fracture, including hip fracture; musculoskeletal. . . in the elderly; growth hormone deficiency in adults or in children; short stature in children; obesity; sleep disorders; cachexia and **protein** loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or burns,. . .

DETD   . . . CBZ-Cl. The reaction was allowed to warm up to Rt and stir overnight. The reaction mixture was poured into aqueous **ammonium chloride** solution and extracted with CH.sub.2 Cl.sub.2. The organic layer was washed with 0.50N HCl solution, dried over MgSO.sub.4 and concentrated.. . .

DETD   . . . 1,2,4-triazole in 10 mL of dry DMF for 3 h. The reaction was cooled to RT and quenched with aqueous **ammonium chloride** solution. The reaction mixture was extracted with ether (3.times.15 mL). The combined organics were washed with brine, dried over Na.sub.2. . .

DETD   . . . described in J. Org. Chem. 1986, 51, 3490) The reaction was stirred for an hour and poured into a saturated **ammonium**

chloride/ammonia solution (1/1) and extracted with ethyl acetate. The organic layer was washed with 1N hydrochloric acid and brine and dried. . .

L15 ANSWER 54 OF 109 USPATFULL
AB Substituted heterocycles of the general structural formula: ##STR1## are tachykinin receptor antagonists useful in the treatment of inflammatory diseases, pain or migraine, asthma, emesis and nausea.
AN 1998:14789 USPATFULL
TI Treatment of migraine with morpholine tachykinin receptor antagonists
IN Dorn, Conrad P., Plainfield, NJ, United States
MacCoss, Malcolm, Freehold, NJ, United States
Hale, Jeffrey J., Westfield, NJ, United States
Mills, Sander G., Woodbridge, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5716942 19980210 <--
AI US 1995-450198 19950525 (8)
RLI Division of Ser. No. US 1994-206771, filed on 4 Mar 1994, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Jarvis, William R. A.
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 6755
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5716942 19980210 <--
SUMM The receptor for substance P is a member of the superfamily of G **protein**-coupled receptors. This superfamily is an extremely diverse group of receptors in terms of activating ligands and biological functions. In addition. . .
SUMM . . . belonging to the tachykinin family of peptides, the latter being so-named because of their prompt contractile action on extravascular smooth **muscle** tissue. The tachykinins are distinguished by a conserved carboxyl-terminal sequence Phe-X-Gly-Leu-Met-NH.sub.2.
SUMM . . . or "parent drug" refers to the biologically active entity that is released via enzymatic action of a metabolic or a **catabolic** process, or via a chemical process following administration of the prodrug. The parent compound may also be the starting material. . .
DETD . . . separated. The organic layer was washed with 100 mL of saturated aqueous sodium bicarbonate solution, 100 mL of saturated aqueous **ammonium chloride** solution, dried over magnesium sulfate and concentrated in vacuo. Crystallization from hexanes at -20.degree. C. for 72 h afforded 8.0. . .
DETD . . . for 15 min and at 25.degree. C. for 15 min. The reaction was quenched with 150 mL of saturated aqueous **ammonium chloride** solution, diluted with 300 mL of ethyl acetate, and the layers were separated. The organic layer was dried over magnesium. . .
DETD . . . and the resulting mixture was stirred cold for 15 min. The reaction was quenched with 50 mL of saturated aqueous **ammonium chloride** solution, diluted with 50 mL of ethyl acetate, and the layers were separated. The organic layer was dried over magnesium. . .
DETD . . . resulting mixture was heated at reflux for 3 h. The reaction was cooled, quenched with 50 mL of saturated aqueous **ammonium**

chloride solution, diluted with 50 mL of ethyl acetate and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . were removed in vacuo and the residue was partitioned between 20 mL of ethyl acetate and 10 mL of saturated **ammonium chloride** solution. The organic layer was separated, dried over sodium carbonate, and concentrated in vacuo. The residue was dissolved in saturated. . .

DETD . . . and the temperature was allowed to rise to 0.degree. C. The reaction was quenched with 100 mL of saturated aqueous **ammonium chloride** solution, transferred to a 1 L flask, and the ether and THF were removed in vacuo. The concentrated mixture was. . .

L15 ANSWER 55 OF 109 USPATFULL

AB The present invention relates to a method to determine the amount of ASP **protein**, a functional derivative or a functional fragment thereof in a plasma sample, wherein the ASP **protein** comprises the following amino acid sequence: ##STR1## wherein the functional derivative comprises at least one selected from the group consisting of one or more amino acid substitution, one or more amino acid deletion and one or more amino acid addition with the proviso that the functional derivative has a biological activity functionally equivalent to ASP, and the functional fragment comprises part of the ASP amino acid sequence and has a biological activity functionally equivalent to ASP; the method comprises the steps of: a) eluting the plasma sample on a column; b) measuring the amount of the ASP **protein**, functional derivative or functional fragment thereof present in said sample by an immunoassay with antibodies specific against one or more sites on C3a. The present invention also relates to the use of any antagonist of the ASP **protein**, functional derivative or functional fragment thereof for the inhibition of triglyceride synthesis in a patient, wherein the antagonist is selected from the group consisting of inhibitors of the alternate complement pathway.

AN 1998:12008 USPATFULL

TI Method of using acylation stimulating **protein**

IN Sniderman, Allan D., Westmount, Canada
Cianflone, Katherine, L'Acadie, Canada

PA McGill University, Montreal, Canada (non-U.S. corporation)

PI US 5714466 19980203 <--

AI US 1994-264022 19940622 (8)

PRAI GB 1993-12819 19930622

DT Utility

FS Granted

EXNAM Primary Examiner: Davenport, Avis M.

LREP Klauber & Jackson

CLMN Number of Claims: 4

ECL Exemplary Claim: 1

DRWN 26 Drawing Figure(s); 13 Drawing Page(s)

LN.CNT 1154

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI Method of using acylation stimulating **protein**

PI US 5714466 19980203 <--

AB The present invention relates to a method to determine the amount of ASP **protein**, a functional derivative or a functional fragment thereof in a plasma sample, wherein the ASP **protein** comprises

the following amino acid sequence: ##STR1## wherein the functional derivative comprises at least one selected from the group consisting.

. method comprises the steps of: a) eluting the plasma sample on a column; b) measuring the amount of the ASP **protein**, functional derivative or functional fragment thereof present in said sample by an immunoassay with antibodies specific against one or more sites on C3a. The present invention also relates to the use of any antagonist of the ASP **protein**, functional derivative or functional fragment thereof for the inhibition of triglyceride synthesis in a patient, wherein the antagonist is selected. . .

SUMM The invention relates to an ASP **protein** involved in the triglyceride synthesis and its uses.

SUMM . . . in both groups, these differences were confirmed and shown to be due to a difference in response to a serum **protein**. Purification of the **protein** was undertaken and a single band on SDS gel electrophoresis obtained. The **protein** had an apparent molecular weight of 14 000, a pI of 9.0 and based on its in vitro activity was named Acylation Stimulating **Protein** (ASP) (Cianflone et al., 1989, J. Biol. Chem. 264(1):426-430).

SUMM . . . of the processes that control the rate at which human adipocytes make triglyceride. However, a metabolic pathway, the Adipsin-Acylation Stimulating **Protein** pathway has recently been described which appears to play a major role in regulating the rate at which this occurs. . . human fibroblasts and adipocytes (Cianflone et al., 1989, J. Biol. Chem. 264(1):426-430). The effector molecule of this pathway, acylation .stimulating **protein** (ASP), is the most potent stimulant yet described of triglyceride synthesis in human adipocytes (Cianflone et al., 1989, J. Biol.. . .

SUMM It would be highly desirable to be provided with a method to determine the amount of ASP **protein** in a plasma sample.

SUMM . . . pathway to a sustained fast with particular regard to the relation between the mobilization of energy from fatty acids and **protein** during this period.

SUMM In accordance with the present invention there is provided a method to determine the amount of ASP **protein**, a functional derivative or a functional fragment thereof in a plasma sample, wherein said ASP **protein** comprises the following amino acid sequence ##STR2## wherein said functional derivative comprises at least one selected from the group consisting. . . said method comprises the steps of (a) eluting said plasma sample on a column; (b) measuring the amount of ASP **protein**, a functional derivative or a functional fragment thereof present in said sample by an immunoassay with antibodies specific against one. . .

SUMM In accordance with the present invention there is also provided for a use of any antagonist or agonist of ASP **protein**, a functional derivative or a functional fragment thereof for the inhibition of triglyceride synthesis in a patient, wherein said ASP **protein** comprises the following amino acid sequence ##STR3## wherein said functional derivative comprises at least one selected from the group consisting. . .

DRWD FIG. 1(A-C) is a graph of **protein** elution at 214 nm for fraction (A) resolved by reverse phase on Vydac.TM. **Protein** C4, (B) by cation exchange on Mono-S HR 5/5.TM. and (C) by reverse phase on .mu.Bondapak.TM. C18;

DRWD . . . 2 is a graph of the biologic activity of in vitro generated C3a

in nmol triglyceride synthesized per mg cell **protein**;
DETD . . . and were obtained from Pharmacia (Uppsala, Sweden). .mu.Bondapak.TM. C18 reverse phase column was obtained from Waters (Millford, Mass.) and Vydac.TM. **Protein** C4 was obtained from the Separations group (Hesperia, Calif.). Trifluoroacetic acid (TFA) was obtained from Chromatographic Specialties Inc. (Brockville, Canada).. . .
DETD . . . the following order: Cation exchange on S-Sepharose.TM. Fast Flow, gel filtration on Sephadex.TM. G-75, reverse phase on a semi-preparative Vydac.TM. **Protein** C4 (1.0.times.25 cm), cation exchange on Mono-S HR 5/5.TM. and reverse phase on .mu.Bondapak.TM. C18 (3.9.times.300 mm). For each chromatographic. . .

the second half of the elution peak monitored by absorbance at 280 nm, were pooled (fraction A, 99.+-.30 mg of **protein**, 274.+-.60 ml, n=8), concentrated and fractionated on G-75. Those tubes with activity were pooled and concentrated to yield fraction B (7.4.+-.3.4 mg of **protein**, 13.+-.1.6 ml, n=8) and stored at -80.degree. C.
DETD Vydac.TM. **Protein** C4
DETD . . . several batches of Fraction B were prepared and pooled (10-50 mg total). The pooled material was loaded on a Vydac.TM. **Protein** C4 column which was eluded with a linear gradient of 25%-65% solvent A (80% ACN) containing 0.1% TFA throughout over 60 minutes at a flow rate of 3 ml/minute and collected as 1 minute fractions with **protein** elution monitored at 214 nm. The biologic activity eluted from this column between 37% to 42% solvent A (fraction C). . .
DETD . . . 0 to 1 M NaCl gradient in solvent B at a flow of 1 ml/minute and fractionated as 0.5 minutes/tube. **Protein** elution was monitored at 214 nm and activity for each fraction tested. The activity which eluted from this column between. . .
DETD . . . column was used as a final purification step and was run using the same solvent system as for the Vydac.TM. **Protein** C4 column. After loading Fraction D on the column, bound **proteins** were eluted over 60 minutes with a linear gradient from 0% to 60% solvent A at a flow rate of. . .
DETD Amino Acid Analysis and **Protein** Quantification
DETD . . . the organic extract were separated and quantified as described previously (Cianflone et al., 1989, J- Biol. Chem. 264(1):426-430). Soluble cell **protein** was dissolved in 0.1 N NaOH and measured by the method of Bradford (Bradford M., 1976, Anal. Biochem. 72:248-254) using a commercial kit (Bio-Rad, Calif.). Bovine serum albumin was used as **protein** standard. Each column fraction was assayed in duplicate or triplicate. Data is expressed as mean.+-.standard deviation.
DETD The purification of ASP is shown in FIG. 1. Fractionated plasma containing small basic **proteins** isolated by ion exchange and gel filtration as previously described by Cianflone et al. (1990, J. Clin. Invest. 85(3):722-730) was. . .
DETD . . . ASP purified from plasma contains 10 Arg as does C3a-desArg, whereas C3a itself contains 11. These results were confirmed when **protein** mass analysis was performed by ion spray ionization. This demonstrated that the mass of the **protein** was 8933.+-.0.3 mass units, .a mass that corresponds not to C3a (9088.7) but to C3a-desArg (8932.5) (Hugli, T. E., 1975,. . .
DETD . . . The results are shown in FIG. 2. The basal level of triglyceride synthesis in the fibroblasts was 29.+-.3.3 nmol/mg cell **protein**. Addition of a partially purified preparation of ASP

increased triglyceride synthesis to 55.+-.6.6 nmol/mg cell
protein, a 87% increase above basal. Addition of a mixture of
B/C3/D resulted in a similar stimulation of triglyceride synthesis as.
. .

DETD . . . in adipose tissue metabolism. They demonstrated that on differentiation murine 3T3 adipocytes express large amounts of a message for a **protein** which they named adipsin (adipocyte trypsin) which is secreted from such cells (Cook et al., 1987, Science 237:402-405). Plasma levels. . .

DETD . . . was considerable homology between the mouse adipsin cDNA sequence and the corresponding amino acid sequence of human factor D, a **protein** integral to the activation of the alternate complement pathway (Cook et al., 1985, Proc. Natl. Acad. Sci. USA, 82:6480-6484). They then isolated a cDNA for human adipsin and showed that it encoded for a **protein** sharing 98% amino acid sequence identity with the **protein** sequence for purified human complement D (White et al., 1992, J. Biol. Chem. 267(13):9210-9213). Most recently, they demonstrated that under. . .

DETD . . . in adipocytes from patients with HyperapoB might be due to reduced responsiveness to ASP, confirming the anabolic character of this **protein** (Teng et al., 1988, J. Physiol., Pharmacol. 66:239-242; Cianflone et al., 1990, J. Clin. Invest. 85(3):722-730; Kwiterovich et al., 1990,. . .

DETD A critical sector of experimental evidence in the present invention is derived from the experiments in which the precursor **proteins**, factors B, D (adipsin) and C3, were incubated under appropriate conditions to generate C3a and added to the medium bathing. . .

DETD . . . represent an adaptive rather than causal finding (Flier et al., 1987, Science 237:405-408). Adipocytes contain message levels for the three **proteins** necessary to generate ASP (Choy et al., 1992, J. Biol. Chem. 267(18):12736-12741). The data of the present invention point, therefore,. . . to the possible existence of what would be a unique regulatory system in which the synthesis of a series of **proteins** then generates a product which by acting on the cell surface alters the essential metabolic property of the cell.

DETD The adipsin-acylation stimulating **protein** system in human adipocytes and the regulation of triacylglycerol synthesis

DETD . . . role. This Example presents the first evidence that this process can be modulated in human adipocytes by the adipsin/Acylation Stimulating **Protein** (ASP) pathway and suggests a novel function for the product of this system, ASP.

DETD . . . respectively at an ASP concentration of 88 ng/mL); 2) when ASP is generated in vitro through incubation of its precursor **proteins** under appropriate conditions, triacylglycerol synthesis increases to the same extent as when plasma-purified ASP is added to the medium; 3) human adipocytes contain mRNA for the specific serine protease, adipsin and the two precursor **proteins**, C3 and factor B, required to interact for the production of ASP; and 4) the extent to which cultured differentiating. . .

DETD . . . by Neri and Frings (Neri & Frings, 1973, Clin. Chem. 19:1201-1202) and expressed as .mu.g triacylglycerol per mg soluble cell **protein**. Cell **proteins** were solubilized on the dishes by addition of 0.1N NaOH and measured by the method of Bradford (Bradford, 1976, Anal.. . .

DETD Acylation Stimulating **Protein** was partially purified from

human plasma as previously described. Complement factors B, C3 and D (Calbiochem, San Diego, Calif.) were. . .

DETD . . . mM MgCl.sub.2, 35 cycles) and complement C3 (10.sup.-5 M TMAC, 2.0 mM MgCl.sub.2, 35 cycles) where TMAC is tetra methyl **ammonium chloride**. Following PCR amplification, samples were separated on a 9% polyacrylamide gel (Laemmli, 1970, Nature 227:680-685) using 0-8% piperazine di-acrylamide as. . .

DETD . . . the indicated concentrations. Triacylglycerol synthesis was measured over a 4 hour period as .sup.3 H-oleate incorporation into triacylglycerol (nmol/mg cell **protein**.+-.standard deviation, n=7 experiments). In the differentiating adipocytes, triacylglycerol synthesis was linear for at least 24 hours, and 100 .mu.M oleate. . . shown). Basal triacylglycerol synthetic rates (shown as 100% on the FIG. 5) were 12.8.+-.2.4 and 65.6.+-.9.3 nmol triacylglycerol/mg soluble cell **protein**/4h.+-.standard deviation in preadipocytes and differentiating adipocytes respectively. *p<0.05, **p<0.01 for % TG in differentiating adipocytes vs. preadipocytes by two mean. . . triacylglycerol synthesis caused by ASP. For example, the basal triacylglycerol synthetic rate in preadipocytes was 12.8.+-.2.4 nmol triacylglycerol/mg soluble cell **protein**/4h (mean.+-.standard deviation), whereas it was 65.6.+-.9.3 nmol triacylglycerol/mg soluble cell **protein**/4h for the differentiating cells. With addition of 88 ng/ml of ASP to the medium, the two absolute rates of synthesis were 20.3.+-.3.0 and 174.0.+-.50.1 nmol triacylglycerol/cell **protein**/4h respectively (168%.+-.11% p<0.0005 and 242%.+-.32% p<0.025 respectively). With higher concentrations of ASP and longer incubation times (24 hours) the ASP. . .

DETD . . . serum albumin. Triacylglycerol synthesis was measured over a 4 hour period as .sup.3 H glucose incorporation into triacylglycerol (nmol/mg cell **protein**); upper panel: preadipocytes with () and without (+) ASP; lower panel: differentiating adipocytes with (o) and without (*) ASP. Reciprocal. . .

DETD . . . would be competent to stimulate triacylglycerol synthesis in human adipocytes. As shown in FIG. 7 when the mixture of precursor **proteins** and specific serine protease is added to cells, it stimulates triacylglycerol synthesis to a degree comparable to that achieved by. . . H-oleate complexed to bovine serum albumin for 4 hours. Triacylglycerol synthesis was measured as described and expressed as nmol/mg cell **protein**/4h.+-.standard deviation for an average of 6 experiments: p not significant for plasma ASP stimulation vs. in vitro generated ASP stimulation. . .

DETD . . . ASP was measured by RIA kit specific for C3a (Amersham, Oakville, Canada) and expressed as ng per mg soluble cell **protein**.+-.standard deviation. * p<0.0005 compared to preadipocytes (two mean t-test). Three types of cells were studied: human skin fibroblasts, human preadipocytes. . .

DETD . . . protease, and factor B and C3, it was examined whether human mature fat cells possess mRNA message for these three **proteins** and compared the findings to human skin fibroblasts which do not produce substantial amounts of ASP in conditioned culture medium.. . .

DETD . . . triacylglycerol synthesis and ASP production was then examined in more detail. For this purpose the mass of triacylglycerol per cell **protein** was taken as an index of differentiation (Hauner et al, 1989, J. Clin. Invest. 84:1663-1670). As shown in FIG. 10,. . . hour incubation time. The cells were extracted and triacylglycerol mass

measured and expressed as nmol triacylglycerol per mg soluble cell **protein**. .sup.3 H-Oleate incorporation into triacylglycerol is expressed as nmol/ mg soluble cell **protein** (TG synthesis). Linear regression correlation: 100 .mu.M oleate: y=0.0349x+34.5 r.sup.2 =0.3621, p<0.0025; 500 .mu.M oleate: y=0.499x+91.7 r.sup.2 =0.545, p<0.0025. The. . .

DETD . . . involved in triacylglycerol synthesis, diacylglycerol acyltransferase, in human fibroblasts and human adipocytes (Yasruel et al, 1991, Lipids 26(7):495-499). A single **protein**, therefore, is able to stimulate two critical processes involved in the construction of a triacylglycerol molecule.

DETD Acylation stimulating **protein** (ASP) is the most potent known stimulant of triglyceride synthesis in human adipocytes. Plasma levels of ASP were measured in. . . ASP levels dropped progressively and were within normal range at the end of the fast (63.+-.16 vs. 53.+-.30 nM/L pNS). **Protein** utilization as evidenced by urine urea nitrogen dropped progressively during the fast, and as plasma ASP levels dropped, there was. . . urine urea nitrogen (r=0.638 p<0.001) indicating that as plasma ASP dropped, energy was mobilized from adipocytes with less utilization of **protein** for this purpose. The data indicate that understanding the role the adipsin-ASP pathway plays in regulation of the rate of. . .

DETD . . . HDL CHOL = HDL cholesterol
FFA = plasma free fatty acids
ApoB = plasma apolipoprotein B
ASP = acylation stimulating **protein**

DETD . . . is to say, the higher the plasma ASP, the higher the urine urea nitrogen, and therefore, the greater the apparent **protein** utilization for energy. Taken together, the data point to increasing utilization of fatty acids but decreasing utilization of **protein** for energy during the fast.

DETD . . . no different from normal. During the fast, as plasma ASP dropped, the adipocytes changed from a markedly anabolic to a **catabolic** status as evidenced by the increase in plasma free fatty acid and ketone levels indicating mobilization of fatty acids from these cells. Furthermore, as plasma ASP dropped, net **protein** breakdown decreased as well, consistent with the hypothesis that obese patients are at a paradoxical disadvantage with respect to energy. . . operate against being able to mobilize energy from adipocytes, and thus necessitate greater mobilization of energy from tissues such as **muscle**. Increased proteolysis and increased **protein** turnover per lean body mass have previously been documented in some studies of obese subjects, though the metabolic basis for. . .

DETD ASP is identical to C3adesarg. C3adesarg is a terminal product of the interaction of the three **proteins** which make up the proximal portion of the alternate complement pathway and was thought to be biologically inactive. However, it. . . of the pathway. Thus, Spiegelman and his colleagues have shown that murine adipocytes contain message for and secrete the three **proteins**, factor B, factor D (or adipsin) and the third component of complement (C3) necessary to produce ASP (Choy et al.,. . .

DETD . . . acids in adipocytes, but the less energy they can mobilize from them, and therefore, the more they must generate from **protein**. Study of the adipsin-ASP pathway may provide, therefore, new approaches

first to the understanding of human obesity and then to. . .

L15 ANSWER 56 OF 109 USPATFULL

AB This invention disclosed (2S,3R)-3(6-aminopurin-9-yl) aralkan-2-ols, a novel class of adenine derivatives (also called 9-aralkyladenines, ARADS), which have been shown to inhibit the enzyme adenosine deaminase at therapeutically useful levels. The relevant inhibitory constant (K.sub.i) values are in the range of 10.sup.-7 -10.sup.-10 M. These compounds with potencies in this range can reversibly inhibit ADA in an effective manner, without permanently deactivating the enzyme. ADA inhibitors that have similar biological profiles have been shown to be of therapeutic value when used to protect heart **muscle** against ischemic damage.

AN 97:123220 USPATFULL

TI Adenosine deaminase inhibitors

IN Abushanab, Elie, Peacedale, RI, United States
Pragnacharyulu, Palle V. P., Bridgton, MO, United States

PA The Board of Governors for Higher Education, State of Rhode Island and Providence Plantations, Providence, RI, United States (U.S. state government)

PI US 5703084 19971230 <--

AI US 1996-680413 19960715 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn

LREP Samuels, Gauthier, Stevens & Reppert

CLMN Number of Claims: 12

ECL Exemplary Claim: 1

DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 543

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5703084 19971230 <--

AB . . . ADA inhibitors that have similar biological profiles have been shown to be of therapeutic value when used to protect heart **muscle** against ischemic damage.

SUMM . . . adenosine deaminase (ADA, also known as adenosine aminohydrolase) is designated as E.C.3.5.4.4. in the international classification system. It is a **catabolic** enzyme which converts adenosine and 2'-deoxyadenosine to the corresponding inosine and 2'-deoxyinosine by replacing the amino group at the sixth. . .

SUMM . . . Biological Evaluation of Putative Metabolites of (+)-erythro-9-(2S-Hydroxy-3R-nonyl) adenine, J. Med. Chem., 1994, 37, 3844, a protective effect on the heart **muscle** against ischemic damage (Abushanab, U.S. Pat. No. 5,491,146 which patent is hereby incorporated by reference in its entirety into this. . .

SUMM These analogs have an additional therapeutic value when used to protect heart **muscle** against ischemic damage. Further, it is believed these analogs have utility in the preservation of organs used for transplants.

DETD . . . was allowed to warm to room temperature and stirred overnight. The reaction was then quenched with 2.times.2 ml saturated aqueous **ammonium chloride**, concentrated under reduced pressure, and diluted with 200 ml of diethyl ether. The ether layer was washed sequentially with 2.times.20. . .

DETD . . . allowed to slowly warm to room temperature and stirred overnight. The reaction was then quenched with 2.times.2 ml saturated aqueous **ammonium chloride**, concentrated under reduced pressure, and diluted with 200 ml of diethyl ether. The ether layer was washed sequentially with 2.times.20. . .

L15 ANSWER 57 OF 109 USPATFULL
AB This invention is directed to the pharmaceutical use of phenyl compounds, which are linked to an aryl moiety by various linkages, for inhibiting tumor necrosis factor. The invention is also directed to the compounds, their preparation and pharmaceutical compositions containing these compounds. Furthermore, this invention is directed to the pharmaceutical use of the compounds for inhibiting cyclic AMP phosphodiesterase.
AN 97:118195 USPATFULL
TI Compounds containing phenyl linked to aryl or heteroaryl by an aliphatic- or heteroatom-containing linking group
IN Palfreyman, Malcolm Norman, Dagenham, United Kingdom
PA Rhone-Poulenc Rorer Limited, Kent, United Kingdom (non-U.S. corporation)
PI US 5698711 19971216 <--
AI US 1995-487377 19950607 (8)
RLI Division of Ser. No. US 1993-98178, filed on 28 Jul 1993
PRAI GB 1991-1777 19910128
GB 1991-17727 19910816
GB 1992-16005 19920728
GB 1992-16006 19920728
GB 1992-16008 19920728
GB 1993-10633 19930521
GB 1993-14847 19930716
DT Utility
FS Granted
EXNAM Primary Examiner: Davis, Zinna Northington
LREP Parker, III, Raymond S., Savitzky, Martin F.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 4763
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5698711 19971216 <--
SUMM . . . compounds, their preparation, pharmaceutical compositions containing these compounds, and their pharmaceutical use in the treatment of disease states associated with **proteins** that mediate cellular activity.
SUMM The principal in vivo actions of TNF can be broadly classified as inflammatory and **catabolic**. It has been implicated as a mediator of endotoxic shock, inflammation of joints and of the airways, immune deficiency states,. . .
SUMM . . . the anti-coagulant activity of vascular endothelial cells. The cachexia associated with certain disease states is mediated through indirect effects on **protein** catabolism. TNF also promotes bone resorption and acute phase **protein** synthesis.
DETD . . . hour, allowed to warm to room temperature and left to stand overnight. The mixture is then quenched with 10% aqueous **ammonium chloride** solution (150 mL), the layers separated and the aqueous layer further extracted with ethyl acetate (2.times.100 mL). The combined organic. . .
DETD . . . and then it is stirred for a further 6 hours. It is then treated with a saturated aqueous solution of **ammonium chloride** (300 mL), and concentrated in vacuo to low volume. The aqueous residue is extracted with ethyl acetate (2.times.200 mL). The. . .
DETD . . . for a further 2 hours in the cold, the mixture is filtered, and the flitrate is washed with saturated aqueous **ammonium chloride** solution. The organic phase is dried over sodium

sulfate and evaporated. The resulting residue is subjected to flash chromatography, eluting. . .

DETD . . . to room temperature and the solution is stirred for a further 2 hours. The reaction mixture is treated with aqueous **ammonium chloride** solution (50 mL) and the solution is extracted with diethyl ether (2.times.200 mL). The combined extracts are dried and concentrated,. . .

DETD . . . 15 minutes, and stirred for a further 1 hour 30 minutes at -75.degree. C. The solution is treated with aqueous **ammonium chloride** solution and extracted with ethyl acetate (3.times.100 mL). The organic layers are combined, washed with brine, dried and concentrated to. . .

DETD . . . 30 minutes. The resulting mixture is then allowed to warm to room temperature overnight, and then treated with saturated aqueous **ammonium chloride** solution (200 mL). The layers are separated and the aqueous layer is further extracted with ethyl acetate (3.times.300 mL). The. . .

DETD . . . is trimmed off and the endothelial layer on the intimal surface is removed by rubbing with a cotton swab. Smooth **muscle** strips are plucked from the aorta and 25 g are homogenized using a Waring Blender in homogenization buffer (20 mM. . .

DETD 3. Effects of Compounds on Tracheal Smooth **Muscle** Contractility.

L15 ANSWER 58 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 97:115291 USPATFULL

TI Steroid receptor modulator compounds and methods

IN Jones, Todd K., Solana Beach, CA, United States
Goldman, Mark E., San Diego, CA, United States
Pooley, Charlotte L.F., San Diego, CA, United States
Winn, David T., San Diego, CA, United States
Edwards, James P., San Diego, CA, United States
West, Sarah J., San Diego, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States
Hamann, Lawrence G., San Diego, CA, United States
Farmer, Luc J., La Jolla, CA, United States
Davis, Robert L., Santee, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)

PI US 5696133 19971209 <--

AI US 1995-465556 19950605 (8)

RLI Continuation-in-part of Ser. No. US 1994-363529, filed on 23 Dec 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn

LREP Jurgensen, Thomas E., Respess, William L., Elmer, James Scott

CLMN Number of Claims: 10

ECL Exemplary Claim: 1

DRWN   No Drawings
LN.CNT 11054
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5696133   19971209   <--
SUMM   . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .
SUMM   . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.
SUMM   . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.
DETD   . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .
DETD   . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .
DETD   . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .
DETD   . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .
DETD   . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone

responsive promoter containing a hormone response element (HRE). This
reporter. . . for the transcription-modulating activity of the
target
IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g, [.sup.3. . . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

DETD . . . concentration (nM), required to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.

DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . . .

L15 ANSWER 59 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating
patients
requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 97:115288 USPATFULL
TI Tricyclic steroid receptor modulator compounds and methods
IN Jones, Todd K., Solana Beach, CA, United States
Winn, David T., San Diego, CA, United States
Goldman, Mark E., San Diego, CA, United States
Hamann, Lawrence G., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States
Farmer, Luc J., La Jolla, CA, United States
Davis, Robert L., Santee, CA, United States
PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)
PI US 5696130 19971209 <--
AI US 1995-462643 19950605 (8)
RLI Continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn
LREP Jurgensen, Thomas E., Respess, William L., Elmer, James Scott
CLMN Number of Claims: 35
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 11334
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5696130 19971209 <--

SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .

DETD . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.

DETD . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.

DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .

DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .

DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .

DETD . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .

DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . for the transcription-modulating activity of the target IR. Thus, the reporter acts as a surrogate for the products (mRNA then

protein) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [3H] steroid (e.g, [.sup.3 H]. . . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

DETD . . . concentration (nM), required to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.

DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . . .

CLM What is claimed is:

. . . . effective in treating ache, modulating male-pattern baldness, treating prostatic hyperplasia, treating prostate cancer or modulating the functioning of the skeletal **muscle** system.

. . . . effective in treating acne, modulating male-pattern baldness, treating prostatic hyperplasia, treating prostate cancer or modulating the functioning of the skeletal **muscle** system.

. . . . effective in treating acne, modulating male-pattern baldness, treating prostatic hyperplasia, treating prostate cancer or modulating the functioning of the skeletal **muscle** system.

L15 ANSWER 60 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 97:115285 USPATFULL

TI Steroid receptor modulator compounds and methods

IN Jones, Todd K., Solana Beach, CA, United States
Zhi, Lin, San Diego, CA, United States
Edwards, James P., San Diego, CA, United States
Tegley, Christopher M., San Diego, CA, United States
West, Sarah J., San Diego, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)

PI US 5696127 19971209 <--

AI US 1995-465429 19950605 (8)

RLI Continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn

LREP Jurgensen, Thomas E., Respess, William L., Elmer, James Scott
CLMN Number of Claims: 36
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 11518
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5696127 19971209 <--
SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .
SUMM . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.
SUMM . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.
DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .
DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .
DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .
DETD . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .
DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced

(co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . for the transcription-modulating activity of the target IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g, [.sup.3. . . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

DETD . . . concentration (nM), required to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.

DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . . .

CLM What is claimed is:

. . . claim 8, wherein the composition comprising a glucocorticoid receptor antagonist of claim 1 is effective in modulating glucocorticoid receptor-mediated carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and functioning of the cardiovascular, kidney, central nervous, immune and skeletal **muscle** systems.

. . . composition according to claim 19, wherein the composition comprising a glucocorticiod receptor antagonist is effective in modulating glucocorticiod receptor-mediated carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and functioning of the cardiovascular, kidney, central nervous, immune and skeletal **muscle** systems.

. . . a patient according to claim 30, wherein the glucocorticoid receptor compound is effective in antagonist modulating glucocorticoid receptor mediated carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and functioning of the cardiovascular, kidney, central nervous, immune and skeletal **muscle** systems.

L15 ANSWER 61 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 97:112477 USPATFULL

TI Steroid receptor modulator compounds and methods

IN Jones, Todd K., Solana Beach, CA, United States

Zhi, Lin, San Diego, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Winn, David T., San Diego, CA, United States
Hamann, Lawrence G., San Diego, CA, United States
Edwards, James P., San Diego, CA, United States
West, Sarah J., San Diego, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)
PI US 5693647 19971202 <--
AI US 1995-464546 19950605 (8)
RLI Continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn
LREP Jurgensen, Thomas E., Respess, William L., Elmer, James Scott
CLMN Number of Claims: 27
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 11185
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5693647 19971202 <--

SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .

SUMM . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-vital agents, particularly in the treatment of exacerbated herpes simplex virus.

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DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . .

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L15 ANSWER 62 OF 109 USPATFULL

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AN 97:112476 USPATFULL

TI Steroid receptor modulator compounds and methods

IN Jones, Todd K., Solana Beach, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States
Edwards, James P., San Diego, CA, United States
West, Sarah J., San Diego, CA, United States

PA   Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S.
       corporation)
PI   US 5693646   19971202   <--
AI   US 1995-464360   19950605 (8)
RLI   Continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994,
       now abandoned
DT   Utility
FS   Granted
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn
LREP   Jurgensen, Thomas E., Respess, William L., Elmer, James Scott
CLMN   Number of Claims: 28
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 11285
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5693646   19971202   <--
SUMM   . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .
SUMM   . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.
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effect of native hormones, and quantifying their activity for
responsive
IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .

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target
IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g, [.sup.3. . .

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DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . .

L15 ANSWER 63 OF 109 USPATFULL

AB The present invention provides novel hydroxamic acids and carbocyclic acids and derivatives thereof and to pharmaceutical compositions and methods of use of these novel compounds for the inhibition of matrix metalloproteinases, such as stromelysin, and inhibit the production of tumor necrosis factor alpha, and for the treatment of arthritis and other related inflammatory diseases. These novel compounds are represented by Formula I below: ##STR1##

AN 97:109937 USPATFULL

TI Hydroxamic and carbocyclic acids as metalloprotease inhibitors

IN Jacobson, Irina Cipora, Boothwyn, PA, United States
Decicco, Carl Peter, Newark, DE, United States
Cherney, Robert Joseph, Newark, DE, United States

PA The DuPont Merck Pharmaceutical Company, Wilmington, DE, United States (U.S. corporation)

PI US 5691381 19971125 <--

AI US 1996-632973 19960416 (8)

RLI Continuation-in-part of Ser. No. US 1995-423192, filed on 18 Apr 1995, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Conrad, Joseph

LREP Kondrad, Karen H.

CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1970
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5691381 19971125 <--
SUMM . . . as rheumatoid and osteoarthritis, corneal, epidermal or gastric
ulceration; tumor metastasis or invasion; periodontal disease and bone disease. Normally these **catabolic** enzymes are tightly regulated at the level of their synthesis as well as at their level of extracellular activity through. . .
SUMM . . . of cartilage degradation in OA (Mankin et al. J. Bone Joint Surg. 52A, 1970, 424-434). There are four classes of **protein** degradative enzymes in mammalian cells: serine, cysteine, aspartic and metalloproteinases. The available evidence supports that it is the metalloproteinases which. . .
SUMM . . . as amino acids which are known to occur biologically in free or
combined form but usually do not occur in **proteins**. Included within this term are modified and unusual amino acids, such as those disclosed in, for example, Roberts and Vellaccio. . .
DETD . . . while the temperature was allowed to rise to -20 .degree. C. Reaction was quenched by addition of excess aqueous saturated **ammonium chloride** solution and concentrated, followed by dilution with water and extraction with ethyl acetate. Organic layer was separated, washed with 5%. . .

L15 ANSWER 64 OF 109 USPATFULL
AB Substituted heterocycles of the general structural formula: ##STR1## are
tachykinin receptor antagonists useful in the treatment of inflammatory diseases, pain or migraine, asthma, and emesis.
AN 97:109895 USPATFULL
TI Morpholine compounds are prodrugs useful as tachykinin receptor antagonists
IN Dorn, Conrad P., Plainfield, NJ, United States
Hale, Jeffrey J., Westfield, NJ, United States
Maccoss, Malcolm, Freehold, NJ, United States
Mills, Sander G., Woodbridge, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5691336 19971125 <--
AI US 1995-525870 19950908 (8)
RLI Continuation-in-part of Ser. No. US 1994-206771, filed on 4 Mar 1994, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Higel, Floyd D.
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 25
ECL Exemplary Claim: 1,24
DRWN No Drawings
LN.CNT 7292
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5691336 19971125 <--
SUMM The receptor for substance P is a member of the superfamily of G **protein**-coupled receptors. This superfamily is an extremely diverse group of receptors in terms of activating ligands and biological
functions. In addition. . .

SUMM . . . belonging to the tachykinin family of peptides, the latter being so-named because of their prompt contractile action on extravascular smooth **muscle** tissue. The tachykinins are distinguished by a conserved carboxyl-terminal sequence Phe-X-Gly-Leu-Met-NH.sub.2. In addition to SP the known mammalian tachykinins include. . .

SUMM . . . or "parent drug" refers to the biologically active entity that is released via enzymatic action of a metabolic or a **catabolic** process, or via a chemical process following administration of the prodrug. The parent compound may also be the starting material. . .

DETD . . . separated. The organic layer was washed with 100 mL of saturated aqueous sodium bicarbonate solution, 100 mL of saturated aqueous **ammonium chloride** solution, dried over magnesium sulfate and concentrated in vacuo. Crystallization from hexanes at -20.degree. C. for 72 h afforded 8.0. . .

DETD . . . for 15 min and at 25.degree. C. for 15 min. The reaction was quenched with 150 mL of saturated aqueous **ammonium chloride** solution, diluted with 300 mL of ethyl acetate, and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . and the resulting mixture was stirred cold for 15 min. The reaction was quenched with 50 mL of saturated aqueous **ammonium chloride** solution, diluted with 50 mL of ethyl acetate, and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . resulting mixture was heated at reflux for 3 h. The reaction was cooled, quenched with 50 mL of saturated aqueous **ammonium chloride** solution, diluted with 50 mL of ethyl acetate and the layers were separated. The organic layer was dried over magnesium. . .

DETD . . . were removed in vacuo and the residue was partitioned between 20 mL of ethyl acetate and 10 mL of saturated **ammonium chloride** solution. The organic layer was separated, dried over sodium carbonate, and concentrated in vacuo. The residue was dissolved in saturated. . .

DETD . . . and the temperature was allowed to rise to 0.degree. C. The reaction was quenched with 100 mL of saturated aqueous **ammonium chloride** solution, transferred to a 1 L flask, and the ether and THF were removed in vacuo. The concentrated mixture was. . .

DETD . . . NMR was taken to confirm reaction completion (see below), then the reaction was quenched into a solution of 6% aqueous **ammonium chloride** (700 mL), maintained at 0.degree.-5.degree.. The organic phase was washed with cold water (3.times.575 mL) and brine (575 mL), then. . .

DETD The reaction was then quenched by addition of a solution of 10% aqueous **ammonium chloride** (20 mL) over 10 min, maintaining the temperature below 10.degree. C. The layers were separated and the organic phase was. . .

DETD . . . NMR was taken to confirm reaction completion (see below), then the reaction was quenched into a solution of 6% aqueous **ammonium chloride** (700 mL), maintained at 0.degree.-5.degree. C. The layers were separated and the organic phase was washed with cold water (3.times.575. . .

DETD . . . 25.degree. C. and aged for 2.5 hours. The batch was diluted with 1:1 hexane:methyl-t-butyl ether (10 L) and 10.9% aqueous **ammonium chloride** (11 L). The phases were partitioned and the aqueous phase was back extracted with 1:1 hexane:methyl-t-butyl ether (2.times.8 L), followed. . .

L15 ANSWER 65 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 97:107096 USPATFULL .

TI Steroid receptor modulator compounds and methods

IN Jones, Todd K., Solana Beach, CA, United States
Goldman, Mark E., San Diego, CA, United States
Pooley, Charlotte L.F., San Diego, CA, United States
Winn, David T., San Diego, CA, United States
Edwards, James P., San Diego, CA, United States
West, Sarah J., San Diego, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States

PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)

PI US 5688810 19971118 <--

AI US 1995-464541 19950605 (8)

RLI Continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn

LREP Jurgensen, Thomas E., Respess, William L., Elmer, James Scott

CLMN Number of Claims: 27

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 11318

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5688810 19971118 <--

SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .

SUMM . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.

SUMM . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and

tissue systems.

DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with saturated **ammonium chloride** (2.times.5 mL). The aqueous layers were back extracted with ethyl acetate (5 mL). The organic layers were combined, dried (Na.sub.2. . . .

DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . . .

DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . . .

DETD . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . . .

DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . . for the transcription-modulating activity of the target IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR. PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g., [.sup.3. . . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

DETD . . . concentration (nM), required to reduce the maximal response by 50%), its agonist potency or EC.sub.50 (nM). PR, AR and GR **protein** binding activity (K.sub.i in nM) is shown in Tables 1-2 and 4.

DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . . .

L15 ANSWER 66 OF 109 USPATFULL

AB Non-steroidal compounds which are high affinity, high selectivity modulators for steroid receptors are disclosed. Also disclosed are

pharmaceutical compositions incorporating such compounds, methods for employing the disclosed compounds and compositions for treating patients requiring steroid receptor agonist or antagonist therapy, intermediates useful in the preparation of the compounds and processes for the preparation of the steroid receptor modulator compounds.

AN 97:107094 USPATFULL
TI Steroid receptor modulator compounds and methods
IN Jones, Todd K., Solana Beach, CA, United States
Winn, David T., San Diego, CA, United States
Zhi, Lin, San Diego, CA, United States
Hamann, Lawrence G., San Diego, CA, United States
Tegley, Christopher M., San Diego, CA, United States
Pooley, Charlotte L. F., San Diego, CA, United States
PA Ligand Pharmaceuticals Incorporated, San Diego, CA, United States (U.S. corporation)
PI US 5688808 19971118 <--
AI US 1995-463231 19950605 (8)
RLI Continuation-in-part of Ser. No. US 1994-363529, filed on 22 Dec 1994, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Huang, Evelyn
LREP Jurgensen, Thomas E., Respess, William L., Elmer, James Scott
CLMN Number of Claims: 27
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 11240
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5688808 19971118 <--
SUMM . . . in the fluid surrounding a cell, pass through the outer cell membrane by passive diffusion and bind to specific IR **proteins** to create a ligand/receptor complex. This complex then translocates to the cell's nucleus, where it binds to a specific gene or genes present in the cell's DNA. Once bound to DNA, the complex modulates the production of the **protein** encoded by that gene. In this regard, a compound which binds an IR and mimics the effect of the native. . .
SUMM . . . antagonists of the present invention can be used to influence the basic, life sustaining systems of the body, including carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, and the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems. In this regard, GR and MR modulators have proved useful in the treatment of. . . and cirrhosis. Accordingly, GR and MR active compounds have been used as immuno stimulants and repressors, wound healing--tissue repair agents, **catabolic**/antianabolic activators and as anti-viral agents, particularly in the treatment of exacerbated herpes simplex virus.
SUMM . . . The GR and MR active compounds and compositions of the present invention will also prove useful as affectors of carbohydrate, **protein** and lipid metabolism, electrolyte and water balance, as well as modulators of the functions of the cardiovascular, kidney, central nervous, immune, skeletal **muscle** and other organ and tissue systems.
DETD . . . as judged by TLC (15% ethyl acetate/hexane). The product mixture was then cooled to room temperature and quenched with saturated **ammonium chloride** (4-5 mL). Ethyl acetate (5 mL) was used to partition the mixture. The organic layer was rinsed with

saturated **ammonium chloride** (2.times.5 mL). The
aqueous layers were back extracted with ethyl acetate (5 mL). The
organic layers were combined, dried (Na.sub.2. . . .

DETD . . . separatory funnel. The organic layer was washed with 2:1 mixture of water and ammonium hydroxide (20 mL) followed by saturated **ammonium chloride** solution (2.times.20 mL) and saturated sodium bicarbonate (20 mL). The aqueous layers were extracted with ether (3.times.10 mL). The organic. . .

DETD . . . The organic layer was washed with 2 to 1 mixture of water and ammonium hydroxide (200 mL) followed by saturated **ammonium chloride** solution (2.times.200 mL) and saturated sodium bicarbonate (200 mL). The aqueous layers were extracted with ether (3.times.100 mL). The organic. . .

DETD . . . partial agonists which mimic, or antagonists which inhibit, the effect of native hormones, and quantifying their activity for responsive IR **proteins**. In this regard, the co-transfection assay mimics an in vivo system in the laboratory. Importantly, activity in the co-transfection assay. . .

DETD . . . into a background cell substantially devoid of endogenous IRs. This introduced gene directs the recipient cells to make the IR **protein** of interest. A second gene is also introduced (co-transfected) into the same cells in conjunction with the IR gene. This second gene, comprising the cDNA for a reporter **protein**, such as firefly luciferase (LUC), controlled by an appropriate hormone responsive promoter containing a hormone response element (HRE). This reporter. . . for the transcription-modulating activity of the target IR. Thus, the reporter acts as a surrogate for the products (mRNA then **protein**) normally expressed by a gene under control of the target receptor and its native hormone.

DETD . . . invention to the steroid receptors was also investigated according to the following methodology for PR and GR, PR and GR **proteins** were prepared from Baculovirus extracts by incorporating the appropriate cDNAs for human progesterone receptor A form (PR-A; P. Kastner et. . .

DETD . . . The final assay volume was 500 .mu.L for PR and 250 .mu.L for GR, and contained .about.5 .mu.g of extract **protein** for PR and .about.50 mg for GR, as well as 2-4 nM of the appropriate [.sup.3 H] steroid (e.g, [.sup.3. . .

DETD To date, binding assays have not been performed utilizing ER or MR **proteins**.

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DETD The gain and loss of sexual organ weights reflect the changes of cell number (DNA content) and cell mass (**protein** content), depending upon the serum androgen concentration. See Y. Okuda et al., 145 J Urol., 188-191 (1991), the disclosure of. . .

L15 ANSWER 67 OF 109 USPATFULL

AB A formulation for IGF-I is disclosed that is useful in treating hyperglycemic disorders and, in combination with growth hormone, in enhancing growth of a mammal. Also disclosed is a process for preparing a formulation of growth hormone and IGF-I from the IGF-I formulation. The IGF-I formulation comprises about 2-20 mg/ml of IGF-I, about 2-50 mg/ml of an osmolyte, about 1-15 mg/ml of a stabilizer, and a buffered solution at about pH 5-5.5, optionally with a surfactant.

AN   97:99265 USPATFULL
TI   Formulated IGF-I Composition
IN   Clark, Ross G., Pacifica, CA, United States
        Yeung, Douglas A., Fremont, CA, United States
        Oeswein, James Q., Moss Beach, CA, United States
PA   Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
PI   US 5681814   19971028   <--
AI   US 1993-71819   19930604 (8)
RLI   Continuation-in-part of Ser. No. US 1991-806748, filed on 13 Dec 1991, now abandoned which is a division of Ser. No. US 1990-535005, filed on 7 Jun 1990, now patented, Pat. No. US 5126324
DT   Utility
FS   Granted
EXNAM   Primary Examiner: Scheiner, Toni R.; Assistant Examiner: Huff, Sheela J.
LREP   Hasak, Janet E.
CLMN   Number of Claims: 8
ECL   Exemplary Claim: 1
DRWN   28 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 2291
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5681814   19971028   <--

SUMM   . . . for example, blood and human cerebral spinal fluid. Most tissues and especially the liver produce IGF-I together with specific IGF-binding **proteins**. These molecules are under the control of growth hormone (GH). Like GH, IGF-I is a potent anabolic **protein**. See Tanner et al., Acta Endocrinol., 84: 681-696 (1977); Uthne et al., J. Clin. Endocrinol. Metab., 39: 548-554 (1974)). IGF-I. . .

SUMM   . . . J. Lab. Clin. Med., 49: 825-836 (1957). Many studies investigating the relationships among GH, IGF-I, cartilage, cultured human fibroblasts, skeletal **muscle**, and growth have supported this somatomedin hypothesis. See, e.g., Phillips and Vassilopoulou-Sellin, N. Engl. J. Med., 302: 372-380; 438-446 (1980);. . .

SUMM   Various methods for formulating **proteins** or polypeptides have been described. These include EP 267,015 published May 11, 1988; EP 308,238 published Mar. 22, 1989; and. . . 193,917 published Sep. 10, 1986, which discloses a slow-release composition of a carbohydrate polymer such as a cellulose and a **protein** such as a growth factor; GB Pat. No. 2,160,528 granted Mar. 9, 1988, describing a formulation of a bioactive **protein** and a polysaccharide; and EP 193,372 published Sep. 3, 1986, disclosing an intranasally applicable powdery pharmaceutical composition containing an active. . . synthetic polymers able to chelate Ca and Mg; and JP 57/026625 published Feb. 12, 1982 disclosing a preparation of a **protein** and water-soluble polymer such as soluble cellulose.

DRWD   . . . the exception of the presence of an N-terminal methionine residue. This added amino acid is a result of the bacterial **protein** synthesis process.

DRWD   . . . has been blocked chemically (i.e., by glucocorticoid treatment) or by a natural condition such as in adult patients or in **catabolic** patients where the IGF-I response to GH is naturally reduced.

DRWD   In addition, the IGF-I is suitably administered together with its

binding **protein**, for example, BP53, which is described in WO 89/09268 published Oct. 5, 1989, which is equivalent to U.S. Ser. No.. . . which are incorporated herein by reference. This administration may be by the method described in U.S. Pat. No. 5,187,151. This **protein** is an acid-stable component of about 53 Kd on a non-reducing SDS-PAGE gel of a 125-150 Kd glycoprotein complex found.

. GH can be delivered coupled to another agent such as an antibody, an antibody fragment, or one of its binding **proteins**.

DRWD . . . their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; **proteins**, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic. . .

DRWD . . . These salts are useful as surface-active germicides for many pathogenic non-sporulating bacteria and fungi and as stabilizers. Examples include octadecyldimethylbenzyl **ammonium chloride**, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride.. . .

DRWD The "inorganic salt" is a salt that does not have a hydrocarbon-based cation or anion. Examples include sodium chloride, **ammonium chloride**, potassium chloride, magnesium chloride, calcium chloride, sodium phosphate, calcium phosphate, magnesium phosphate, potassium phosphate, ammonium phosphate, sodium sulfate, ammonium sulfate,. . .

DRWD . . . the final pH will not vary significantly from 5.4 in the final IGF-I/GH mixture to maintain good solubility of both **proteins** over a wide mixing ratio range. However, a broader pH range in terms of stability of both **proteins** is from about 5 to about 6.

DRWD . . . associated with aging such as increasing lean mass to fat ratios, immuno-deficiencies including increasing CD4 counts and increasing immune tolerance, **catabolic** states associated with wasting, etc., Laron dwarfism, insulin resistance, and so forth.

DETD . . . mixed with hGH in dose ratios of IGF-I:hGH of greater than about 2:1 to provide a stable co-mix of both **proteins**. In this example, the IGF-I formulation used to achieve this was:

DETD . . . from the blood. The IGF-I concentration in the plasma samples was measured after acid-ethanol extraction to remove the IGF binding **proteins**) by radioimmunoassay.

DETD . . . as much (230-250 grams) as the dw/dw rats, and might be expected to have higher concentrations of plasma IGF binding **proteins**, the doses of IGF-I were doubled, compared to those used in the earlier examples in the dw/dw rat.

L15 ANSWER 68 OF 109 USPATFULL

AB This invention is directed to the pharmaceutical use of phenyl compounds, which are linked to an aryl moiety by various linkages, for inhibiting tumor necrosis factor. The invention is also directed to the compounds, their preparation and pharmaceutical compositions containing these compounds. Furthermore, this invention is directed to the pharmaceutical use of the compounds for inhibiting cyclic AMP phosphodiesterase.

AN 97:96889 USPATFULL

TI Compounds containing phenyl linked to aryl or heteroaryl by an aliphatic-or heteroatom-containing linking group

IN Fenton, Garry, Dagenham, United Kingdom
Morley, Andrew David, Dagenham, United Kingdom
Palfreyman, Malcolm Norman, Dagenham, United Kingdom
Ratcliffe, Andrew James, Dagenham, United Kingdom

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       Sharp, Brian William, Dagenham, United Kingdom
       Stuttle, Keith Alfred James, Dagenham, United Kingdom
       Thurairatnam, Sukanthini, Dagenham, United Kingdom
       Vacher, Bernard Yvon Jack, Dagenham, United Kingdom
PA     Rhone-Poulenc Rorer Limited, Kent, England (non-U.S. corporation)
PI     US 5679696              19971021                          <--
AI     US 1995-484805          19950607 (8)
RLI    Division of Ser. No. US 1993-98178, filed on 28 Jul 1993
PRAI   GB 1992-15989       19920728
       GB 1992-16764       19920807
       GB 1993-10938       19930527
       GB 1993-11281       19930601
DT     Utility
FS     Granted
EXNAM  Primary Examiner: Davis, Zinna Northington
LREP   Parker, III, Raymond S., Savitzky, Martin F., Baker, R. Keith
CLMN   Number of Claims: 27
ECL    Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 4777
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI     US 5679696              19971021                          <--
SUMM   . . . compounds, their preparation, pharmaceutical compositions
       containing these compounds, and their pharmaceutical use in the
       treatment of disease states associated with proteins that
       mediate cellular activity.
SUMM   The principal in vivo actions of TNF can be broadly classified as
       inflammatory and catabolic. It has been implicated as a
       mediator of endotoxic shock, inflammation of joints and of the airways,
       immune deficiency states,. . .
SUMM   . . . the anti-coagulant activity of vascular endothelial cells. The
       cachexia associated with certain disease states is mediated through
       indirect effects on protein catabolism. TNF also promotes bone
       resorption and acute phase protein synthesis.
DETD   . . . hour, allowed to warm to room temperature and left to stand
       overnight. The mixture is then quenched with 10% aqueous
       ammonium chloride solution (150 mL), the layers
       separated and the aqueous layer further extracted with ethyl acetate
       (2.times.100 mL). The combined organic. . .
DETD   . . . and then it is stirred for a further 6 hours. It is then
       treated with a saturated aqueous solution of ammonium
       chloride (300 mL), and concentrated in vacuo to low volume. The
       aqueous residue is extracted with ethyl acetate (2.times.200 mL). The.
       . .
DETD   . . . for a further 2 hours in the cold, the mixture is filtered,
and
       the filtrate is washed with saturated aqueous ammonium
       chloride solution. The organic phase is dried over sodium
       sulfate and evaporated. The resulting residue is subjected to flash
       chromatography, eluting. . .
DETD   . . . to room temperature and the solution is stirred for a further
2
       hours. The reaction mixture is treated with aqueous ammonium
       chloride solution (50 mL) and the solution is extracted with
       diethyl ether (2.times.200 mL). The combined extracts are dried and
       concentrated,. . .
DETD   . . . 15 minutes, and stirred for a further 1 hour 30 minutes at
       -75.degree. C. The solution is treated with aqueous ammonium
       chloride solution and extracted with ethyl acetate (3.times.100
       mL). The organic layers are combined, washed with brine, dried and
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concentrated to. . .

DETD . . . 30 minutes. The resulting mixture is then allowed to warm to room temperature overnight, and then treated with saturated aqueous **ammonium chloride** solution (200 mL). The layers are separated and the aqueous layer is further extracted with ethyl acetate (3.times.300 mL). The. . .

DETD . . . is trimmed off and the endothelial layer on the intimal surface is removed by rubbing with a cotton swab. Smooth **muscle** strips are plucked from the aorta and 25 g are homogenized using a Waring Blender in homogenization buffer (20 mM. . .

DETD 3. Effects of compounds on tracheal smooth **muscle** contractility.

L15 ANSWER 69 OF 109 USPATFULL

AB Disclosed are CHO cells which are capable of continued production of human LH-RH receptor **proteins**, or cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or partial peptides thereof; methods for screening compounds which have affinity for an LH-RH receptor by contacting the compound with the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the partial peptides thereof; kits for screening them; the compounds which have affinity for the LH-RH receptor obtained by methods for the screening or kits for the screening; and pharmaceutical compositions containing the compound, thereby being able to early provide prophylactic or therapeutic compositions, for example, for prostate cancer, uterine cancer, breast cancer, a pituitary tumor, endometriosis, hysteromyoma or precocious puberty. They are also useful as a pregnancy controlling composition such as contraceptive or a menstrual cycle controlling composition.

AN 97:94117 USPATFULL

TI CHO cells that express human LH-RH receptor

IN Onda, Haruo, Ibaraki, Japan
Ohkubo, Shoichi, Ibaraki, Japan
Hinuma, Shuji, Ibaraki, Japan
Sawada, Hidekazu, Osaka, Japan

PA Takeda Chemical Industries, Ltd., Osaka, Japan (non-U.S. corporation)

PI US 5677184 19971014 <--

AI US 1995-423691 19950418 (8)

PRAI JP 1994-80731 19940419
JP 1994-218349 19940913

DT Utility

FS Granted

EXNAM Primary Examiner: Allen, Marianne P.; Assistant Examiner: Hayes, Robert C.

LREP Conlin, David G., Resnick, David S.Dike, Bronstein Roberts & Cushman, LLP

CLMN Number of Claims: 2

ECL Exemplary Claim: 1

DRWN 6 Drawing Figure(s); 4 Drawing Page(s)

LN.CNT 2214

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5677184 19971014 <--

AB Disclosed are CHO cells which are capable of continued production of human LH-RH receptor **proteins**, or cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or partial peptides thereof; methods for screening compounds which have affinity for an LH-RH receptor by contacting the compound with the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH

receptor **proteins** or the partial peptides thereof; kits for screening them; the compounds which have affinity for the LH-RH receptor obtained by. . .

SUMM The present invention relates to Chinese Hamster Ovary (CHO) cells having ability to continue producing human LH-RH (luteinizing hormone-releasing) receptor **proteins**, or cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or peptide fragments thereof; methods for screening a compound or a salt thereof which has affinity for an LH-RH receptor by using the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the peptide fragments thereof; kits for screening a compound or a salt thereof which has affinity for an LH-RH. . .

SUMM . . . is therefore considered that COS7 cells are unsuitable for screening use. Use of human pituitary fractions as human LH-RH receptor **protein** have been considered. However, human-derived tissues are very difficult to be obtained, resulting in unsuitableness for screening use.

SUMM . . . is to provide CHO cells having ability to express human LH-RH receptor, cell membrane fractions thereof; recombinant human LH-RH receptor **proteins** or peptide fragments thereof; methods for screening a compound or a salt thereof which has affinity for an LH-RH receptor by using the CHO cells or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the peptide fragments thereof; kits for screening compounds or a salt thereof which has affinity for an LH-RH receptor.. . .

SUMM (1) A CHO cell containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA, and wherein said cell is capable of continued production of a recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**;

SUMM (2) A CHO cell containing a recombinant human LH-RH receptor **protein**, which is produced by cultivating the CHO cell described in (1) under conditions such that the recombinant human LH-RH receptor **protein** is continuously expressed from a DNA coding for a human LH-RH receptor **protein**, or a cell membrane fraction thereof;

SUMM (4) A recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which is isolated from the CHO cell described in (2);

SUMM (5) A method for producing a recombinant human LH-RH receptor **protein**, which comprises cultivating the CHO cell described in (1) under conditions suitable for expression of the recombinant human LH-RH receptor, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and wherein said cell is capable of continued production of a recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**;

SUMM . . . salt thereof which has affinity for an LH-RH receptor, which comprises contacting the compound with the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof described in (4) and measuring the affinity of said compound for the. . .

SUMM . . . which contains the CHO cell or the cell membrane fraction thereof described in (2), or the recombinant human LH-RH receptor

protein, the peptide fragment thereof or a salt thereof described in (4);

SUMM (22) The CHO cell described in (1) or (2), in which the DNA coding for the human LH-RH receptor **protein** is a DNA containing a DNA fragment having a nucleotide sequence represented by SEQ ID NO: 1;

SUMM (23) The recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof described in (4), in which said recombinant human LH-RH receptor **protein** is a **protein** having an amino acid sequence represented by SEQ ID NO: 2, an amino acid sequence lacking one amino acid or. . . amino acid sequence represented by SEQ ID NO: 2 are substituted by another amino acid or other amino acids, a **protein** in which an N-terminal signal peptide of said **protein** is removed, a **protein** in which a side chain of an amino acid in a molecule of said **protein** is protected with an appropriate protective group (for example, a C.sub.1-6 acyl group such as formyl or acetyl), or a **protein** in which a sugar chain is bound to said **protein** ;

SUMM . . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of guanine nucleotide refulatory **proteins** (G **proteins**) and cell growth);

SUMM . . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of guanine nucleotide regulatory **proteins** (G **proteins**) and cell growth);

SUMM (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a ligand to an LH-RH receptor, and

SUMM (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a ligand to an LH-RH receptor and a. . .

SUMM (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a labeled ligand to an LH-RH receptor, and

SUMM (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof described in (4) with a labeled ligand to an LH-RH receptor and. . .

SUMM (30) A vector containing a DNA coding for a human LH-RH receptor **protein** which expresses a human LH-RH receptor **protein** , which is designated pA1-11/hLH-RHR contained in Escherichia coli MV1184//pA1-11/hLH-RHR(FERM BP-4645, IFO 15812);

SUMM . . . CHO cell described in any one of (1)-(3) and (20)-(22), in which the DNA coding for the human LH-RH receptor **protein** is the expression vector described in (30);

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein**, continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which comprises adaptating the CHO cell described in (1) to an agitation culture in suspension by use of a

serum-containing. . .

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein,** continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein,** which comprises adaptating the CHO cell described in (1) to an agitation culture in suspension by use of a serum-containing. . .

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein,** continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein,** which comprises adaptating the CHO cell described in (1) to a serum-free medium with a gradual decrease in serum concentration. . .

SUMM . . . producing the CHO cell capable of suspension culture described in (3) containing a DNA coding for a human LH-RH receptor **protein,** continuously expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein,** which comprises adaptating the CHO cell described in (1) to a serum-free culture in static culture (for example, plate culture),. . .

SUMM (36) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein,** constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein,** which comprises adaptating a cell containing the DNA coding for the receptor **protein,** constitutively expressing a recombinant receptor **protein** from said DNA and wherein said cell is capable of continued production of the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to an agitation culture in suspension by use of a serum-containing medium;

SUMM (37) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein,** constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein,** which comprises adaptating a cell containing the DNA coding for the receptor **protein,** constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, followed by adaptating to a serum-free medium, or

SUMM (38) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein,** constitutively expressing a recombinant receptor **protein** from

said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises adaptating a cell containing the DNA coding for the human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant human-derived receptor **protein** having activities substantially equivalent to those of the natural human-derived receptor **protein** to a serum-free medium with a gradual decrease in serum concentration under agitation culture in suspension;

SUMM (39) A method for producing a cell capable of suspension culture containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to a serum-free culture in static culture (for example, plate culture), followed by adaptating to an agitation culture in suspension;. . .

SUMM . . . capable of proliferation in suspension produced by the method described in (36)-(39), which contains a DNA coding for a receptor **protein**, constitutively expresses a recombinant receptor **protein** from said DNA and has ability to keep producing a recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**.

SUMM As used herein, the "recombinant human LH-RH receptor **protein**" is a **protein**, mutein or peptide fragment having biological activities substantially equivalent to those of the natural human LH-RH receptor **protein**. Substantially equivalence will depend on the particular activity one is looking at. Biological activities include, for example, ligand binding and. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth.

DRWD FIG. 1 shows a nucleotide sequence of cDNA coding for a human LH-RH receptor **protein** prepared in Reference Example 2;

DRWD FIG. 3 is a schematic representation showing the construction of a human

LH-RH receptor **protein** expression vector designated pA1-11/hLH-RHR, wherein Amp.sup.r represents an ampicillin resistant gene, DHFR represents a dihydrofolate reductase gene, and SV40ori represents. . .

DETD The CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA, wherein said cell is capable of continued production of the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** is the CHO cell into which the expression vector containing the DNA coding for the human LH-RH receptor **protein** is introduced.

DETD As the DNA coding for the human LH-RH receptor **protein**, for example, cDNA or genomic DNA coding for the human LH-RH receptor **protein** is used. However, it is not necessarily limited thereto

as long as it has a nucleotide sequence coding for the human LH-RH receptor **protein** or a peptide fragment thereof having ligand binding activities substantially equivalent to those of the human LH-RH receptor **protein**. For example, although known cDNA or genomic DNA coding for the human LH-RH receptor **protein** can be used, synthetic DNA may also be used. Examples thereof include DNA having the nucleotide sequence represented by SEQ. . . . of the 54th to 1037th nucleotides of the nucleotide sequence shown in FIG. 1) coding for a human LH-RH receptor **protein** having the amino acid sequence represented by SEQ ID NO: 2 (FIG. 2). Specifically, cDNA having the nucleotide sequence of. . . .

DETD In order to introduce the DNA fragment coding for the human LH-RH receptor **protein** into the CHO cell to express the recombinant human LH-RH receptor **protein**, it is necessary to construct the expression vector.

DETD . . . which the above-mentioned promoter (particularly, the SR.alpha. promoter) is inserted upstream from the DNA coding for the human LH-RH receptor **protein**, the polyadenylation signal is inserted downstream from the DNA coding for the human LH-RH receptor **protein**, further, the above-mentioned promoter (particularly, the SV40 promoter), the DHFR gene and/or the polyadenylation signal is inserted downstream therefrom, and. . . .

DETD . . . preferred in which the SV40ori and SR.alpha. promoters are inserted upstream from the DNA coding for the human LH-RH receptor **protein** in vector pA1-11, the polyadenylation signal is inserted downstream from the DNA coding for the human LH-RH receptor **protein**, further, the SV40 promoter, the DHFR gene and the polyadenylation signal in this order are inserted downstream therefrom, and the. . . .

DETD . . . can also be used. The animal cells may be any as long as they can express the human LH-RH receptor **proteins**. Examples thereof include 293 cells, Vero cells, L cells, myeloma cells, C127 cells, BALB3T3 cells and Sp-2/0 cells. Of these,. . . .

DETD The CHO cell containing the recombinant human LH-RH receptor **protein** of the present invention can be produced by cultivating the CHO cell containing the DNA coding for the human LH-RH receptor-**protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA, wherein said cell is capable of continued production of the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein**, under such conditions that the DNA coding for the human LH-RH receptor **protein** can be constitutively expressed.

DETD Methods for stably expressing the human LH-RH receptor **proteins** using the CHO cells described above include methods of selecting the CHO cells by clone selection in which the above-mentioned. . . .

DETD . . . using the selection markers make it possible to obtain stable cell lines having high expression of the human LH-RH receptor **proteins**. Furthermore, when the DHFR genes are used as the selection marker, cultivation can also be performed with a gradual increase. . . .

DETD . . . method for producing a CHO cell capable of proliferation in suspension containing a DNA coding for a human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA, wherein said cell is capable of continued production of the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of a natural human LH-RH receptor **protein**, which comprises

DETD (1) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, or

DETD (2) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, followed by adaptating to a serum-free medium, or

DETD (3) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to a serum-free medium with a gradual decrease in serum concentration under an agitation culture in suspension, or

DETD (4) adaptating a CHO cell of the present invention containing the DNA coding for the human LH-RH receptor **protein**, constitutively expressing a recombinant human LH-RH receptor **protein** from said DNA and having ability to continue producing the recombinant human LH-RH receptor **protein** having activities substantially equivalent to those of the natural human LH-RH receptor **protein** to a serum-free culture in static culture (for example, plate culture), followed by adaptating to an agitation culture in suspension.

DETD . . . selection drugs such as MTX to make them selection drug-resistant, thereby amplifying the structural genes of the human LH-RH receptor **proteins**, or to improve productivity at the line level by combining them.

DETD Using the thus-obtained highly-productive CHO cell lines for the human LH-RH receptor **proteins**, large-scale cultivation is conducted to produce the target human LH-RH receptor **proteins** in large amounts. Culture apparatuses used in this case include known agitation culture tanks equipped with elements necessary for cultivation. . . . means as so desired (Shin Seikagaku Jikken Koza (Course of Biochemical Experiments, New Series), 1, edited by Nippon Seikagaku Kai, **Proteins** VI, Synthesis and Expression, pages 282 and 286, Tokyo Kagaku Dojin (1992); Shin Seikagaku Jikken Koza (Course of Biochemical Experiments,. . .

DETD The cell containing the recombinant human LH-RH receptor **protein** can be produced from the cell containing the expression vector bearing the DNA coding for the human LH-RH receptor **protein** in the manner as described above.

DETD Examples of the cells which can highly express the DNAs coding for the human LH-RH receptor **proteins** in the present invention include the CHO(dhfr.sup.-) cell containing the expression vector designated pA1-11/hLH-RHR which is obtained in Example 1. . . . CHO/L39-7 is preferred. Further, examples of the cells which can highly express the DNAs coding for the human LH-RH receptor **proteins** and can be suspension cultivated include CHO(dhfr.sup.+) cells designated CHO/LS and CHO/LH-8. Of these, the CHO(dhfr.sup.+) cell designated CHO/LH-8 is. . .

DETD . . . CHO(dhfr.sup.-) cells have receptor activities (for example, ligand binding activity) about 10 times higher than the recombinant human LH-RH receptor **protein**-containing COS-7 cells. Expression of receptor in COS-7 cells is transient but expression of receptor in CHO cells is continuous. Accordingly,. . .

DETD . . . for producing the CHO cells capable of proliferation in suspension can be applied not only to the human LH-RH receptor **proteins**, but also to all receptor **proteins**, and can be applied not only to the CHO cells, but also to all cells.

DETD (1) a method for producing a cell capable of proliferation in suspension containing a DNA coding for a human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing a recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**, which comprises

DETD (i) adaptating a cell containing the DNA coding for the human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant human-derived receptor **protein** having activities substantially equivalent to those of the natural human-derived receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, or

DETD (ii) adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant human-derived receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to an agitation culture in suspension by use of a serum-containing medium, followed by adaptating to a serum-free medium, or

DETD (iii) adaptating a cell containing the DNA coding for the human-derived receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to a serum-free medium with a gradual decrease in serum concentration under an agitation culture in suspension, or

DETD (iv) adaptating a cell containing the DNA coding for the receptor **protein**, constitutively expressing a recombinant receptor **protein** from said DNA and having ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of the natural receptor **protein** to a serum-free culture in static culture (for example, plate culture), followed by adaptating to an agitation culture in suspension;. . .

DETD . . . capable of proliferation in suspension produced by the method described in (1), which contains a DNA coding for a receptor **protein**, constitutively expresses a recombinant receptor **protein** from said DNA and has ability to continue producing the recombinant receptor **protein** having activities substantially equivalent to those of a natural receptor **protein**.

DETD The receptor **proteins** are not limited to the human LH-RH receptor **proteins**. They may be either known or novel receptor **proteins**. Examples thereof include endothelin receptor **proteins**, TRH receptor **proteins**, PACAP receptor **proteins**, histamine receptor **proteins**, somatostatin receptor **proteins**, CRF receptor **proteins**, neurotensin receptor **proteins**, IL-8 receptor **proteins**

, galanin receptor **proteins**, GHRH receptor **proteins**, prostaglandin E.sub.2 receptor **proteins**, prostaglandin I.sub.2 receptor **proteins**, bradykinin receptor **proteins**, CNP receptor **proteins**, CC chemokine receptor **proteins**, angiotensin receptor **proteins**, bombesin receptor **proteins**, kanabinoid receptor **proteins**, cholecystokinin receptor **proteins**, glutamine receptor **proteins**, serotonin receptor **proteins**, melatonin receptor **proteins**, neuropeptide Y receptor **proteins**, opioid receptor **proteins**, purine receptor **proteins**, vasopressin receptor **proteins**, oxytocin receptor **proteins**, VIP (Vasoactive intestinal and related peptide) receptor **proteins**, dopamine receptor **proteins**, motilin receptor **proteins**, amylin receptor **proteins**, bradykinin receptor **proteins**, CGRP (calcitonin gene related peptide) receptor **proteins**, leukotriene receptor **proteins**, pancreastatin receptor **proteins**, thromboxane receptor **proteins**, adenosine receptor **proteins**, adrenalin receptor **proteins**, GRO.alpha. receptor **proteins**, GRO.beta. receptor **proteins**, GRO.gamma. receptor **proteins**, NAP-2 receptor **proteins**, ENA-78 receptor **proteins**, PF-4 receptor **proteins**, IP10 receptor **proteins**, GCP-2 receptor **proteins**, MCP-1 receptor **proteins**, HC14 receptor **proteins**, MCP-3 receptor **proteins**, I-309 receptor **proteins**, MIP1.alpha. receptor **proteins**, MIP-1.beta. receptor **proteins**, RANTES receptor **proteins**, enterogastrine receptor **proteins**, pancreatic polypeptide receptor **proteins** and adrenomedulin receptor **proteins**.

DETD The DNAs coding for these receptor **proteins** can be cloned by methods well known in the art or methods based thereon. Expression vectors containing the DNAs can. . . . methods based thereon, specifically according to methods for constructing expression vectors containing the DNAs expressing the above-mentioned human LH-RH receptor **proteins**.

DETD . . . the CHO cells. Any cells may be used as long as they can express the DNAs coding for the receptor **proteins** (preferably, human-derived receptor **proteins**) and can produce the recombinant receptor **proteins** having activities substantially equivalent to those of the natural receptor **proteins**. For example, they include Escherichia, Bacillus, yeast, insects and animal cells.

DETD . . . proliferation in suspension, methods for cultivating the cells capable of proliferation in suspension and methods for isolating the recombinant receptor **proteins** produced, methods similar to those used for the above-mentioned human LH-RH receptor **proteins** can be employed.

DETD The cell membrane fraction of the cell (for example, the CHO cell) containing the recombinant human LH-RH receptor **protein** of the present invention means a fraction rich in the cell membrane content which is obtained by methods well known in the art after disruption of the cell containing the recombinant human LH-RH receptor **protein** of the present invention. Methods for disrupting the cell include crushing of the cell with a homogenizer and disruption with. . . . as a

membrane fraction. The membrane fraction contains a large amount of membrane components such as the human LH-RH receptor **protein**, cell-derived phospholipids and membrane **proteins**.

DETD The amount of the human LH-RH receptor **proteins** in the cells containing the recombinant human LH-RH receptor **proteins** of

the present invention or the cell membrane fractions thereof is preferably about 0.01 to about 100 pmol per 1 mg of the membrane **protein**, or preferably 10.sup.3 to 10.sup.8 molecules per cell, and more preferably 10.sup.4 to 10.sup.6 molecules per cell. The larger expression. . .

DETD Examples of the recombinant human LH-RH receptor **proteins** of the present invention include the recombinant human LH-RH receptor **protein** having the amino acid sequence represented by SEQ ID NO: 2 which is produced by expressing the DNA having the nucleotide sequence represented by SEQ ID NO: 1. They further include the **protein** having the amino acid sequence lacking one amino acid or two or more amino acids in the amino acid sequence represented by SEQ ID NO: 2, the **protein** having the amino acid sequence in which one amino acid or two or more amino acids are added to the. . . ID NO: 2 are substituted by another amino acid or other amino acids. Further, in these recombinant human LH-RH receptor **proteins**, N-terminal signal peptides may be cleaved, side chains of amino acids in molecules may be protected with appropriate protective groups (for example, C.sub.1-6 acyl groups such as formyl and acetyl), or sugar chains may be bound to the **proteins**.

DETD Recombinant human LH-RH receptor **proteins** of the present invention may be different from the known human LH-RH receptor **proteins** such as natural human LH-RH receptor **protein**, a recombinant human LH-RH receptor **protein** produced by cultivating COS-7 cells containg a DNA coding for human LH-RH receptor **protein**, in the kind, size and/or numbers of the glycosyl chains. Thus, the molecular weight of the recombinant human LH-RH receptor **protein** may be different from the molecular weight of the known human LH-RH receptor **proteins**.

DETD As the salts of the recombinant human LH-RH receptor **proteins** of the present invention, pharmaceutically acceptable acid addition salts are preferred among others. Examples of such salts include salts with. . .

DETD The recombinant human LH-RH receptor **protein** of the present invention can be produced, for example, by cultivating the CHO cell of the present invention containing the vector bearing the DNA coding for the human LH-RH receptor **protein**, under such conditions that the DNA coding for the human LH-RH receptor **protein** can be expressed. The recombinant human LH-RH receptor **protein** can be isolated from the resulting cell containing the recombinant human LH-RH receptor **protein**, for example, according to the following methods.

DETD When the recombinant human LH-RH receptor **protein** is extracted from the cells, the cells are collected by known methods after cultivation, and suspended in an appropriate buffer. . . a homogenizer or freeze-thawing, followed by centrifugation or filtration to obtain a crude extract of the recombinant human LH-RH receptor **protein**.

DETD . . . such as CHAPS, digitonin or Triton X-100 (registered trade mark, hereinafter occasionally abbreviated as "TM"). The recombinant human LH-RH receptor **protein** contained in the resulting extract can be purified by suitable combinations of the separating-purifying methods well known in the art.. . .

DETD When the recombinant human LH-RH receptor **proteins** thus obtained are free forms, they can be converted to appropriate salts by known methods or methods based thereon. Conversely, when the **proteins** are obtained in the salt state, they can be converted to the free forms or other salts by known methods. . .

DETD Before or after purification, the recombinant human LH-RH receptor **protein** can be modified with an appropriate **protein** modifying enzyme to arbitrarily modify the **protein** or to partially eliminate a polypeptide therefrom. The **protein** modifying enzymes used include trypsin, chymotrypsin, arginyl endopeptidase, **protein** kinase and glucosidase.

DETD The recombinant human LH-RH receptor **protein** produced by cultivating the CHO cell containing the vector bearing the DNA coding for the human LH-RH receptor **protein**, under the conditions that the DNA coding for the human LH-RH receptor **protein** can be expressed, as described above, has activities substantially equivalent to those of the natural human LH-RH receptor **protein** . The substantially equivalent activities include, for example, ligand binding activity and signal information transmission. The ligand binding activity includes binding. . . LH-RH), LH-RH receptor superagonist (e.g. leuprorelin, leuprorelin acetate) or LH-RH receptor antagonist. As used herein, the "recombinant human LH-RH receptor **protein**" is a **protein**, mutein or peptide fragment having biological activities substantially equivalent to those of the natural human LH-RH receptor **protein**. Substantially equivalence will depend on the particular activity one is looking at. Biological activities include, for example, ligand binding and. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth. The ligand binding activity includes binding with, for example, LH-RH receptor agonist (e.g. LH-RH), LH-RH receptors superagonist (e.g. leuprorelin, leuprorelin acetate) or LH-RH receptor antagonist. Preferably, the recombinant human LH-RH receptor **protein** will have at least two of these activities, most preferably at least three. In addition, the recombinant receptor **protein** will have at least 50% of the activity of the natural human LH-RH receptor **protein**, preferably at least 70%, most preferably at least 90%. Accordingly, quantitative factors such as the molecular weight of the receptor **protein** may be different.

DETD As the peptide fragments of the recombinant human LH-RH receptor **proteins** of the present invention, for example, a site exposed outside the cell membranes is used. Specifically, the peptide fragment is. . .

DETD As the salts of the peptide fragments of the recombinant human LH-RH receptor **proteins** of the present invention, pharmaceutically acceptable acid addition salts are preferred among others. Examples of such salts include salts with. . .

DETD The peptide fragments of the recombinant human LH-RH receptor **proteins** of the present invention or a salt thereof can be produced by peptide synthesis well known in the art or by cleaving the recombinant human LH-RH receptor **proteins** of the present invention with appropriate peptidases. For example, either solid phase synthesis methods or liquid phase synthesis methods may. . . the peptides. Namely, the target peptides can be produced by condensing peptide fragment(s) or amino acid(s) which can constitute the **proteins** of the present invention with residual moieties, and eliminating protective groups when the products have the protective groups. Known condensing. . .

DETD (4) H. Yazima, S. Sakakibara et al., Seikagaku Jikken Koza (Course of Biochemical Experiments), 1, Chemistry of **Proteins** IV, 205 (1977); and

DETD . . . by the above-mentioned methods are free forms, they can be converted to appropriate salts by known methods. Conversely, when the **proteins** are obtained in the salt state, they can be converted to the free forms by known methods.

DETD The CHO cells containing the recombinant human LH-RH receptor **proteins** or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins**, the peptide fragments thereof or a salt thereof according to the present invention is useful for screening a compound or. . .

DETD (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a ligand to an LH-RH receptor, and

DETD (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a ligand to an LH-RH receptor and. . .

DETD . . . to an LH-RH receptor with the CHO cell or the cell membrane fraction thereof containing the recombinant human LH-RH receptor **protein**, and

DETD . . . and a test compound with the CHO cell or the cell membrane fraction thereof containing the recombinant human LH-RH receptor **protein**.

DETD . . . the present invention comprises measuring the binding of a ligand to an LH-RH receptor to the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof, or the CHO cell containing the recombinant human LH-RH receptor **protein** or the cell membrane fraction thereof, for both the cases of (i) and (ii), or measuring cell stimulation activities, followed. . .

DETD (i) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a labeled ligand to an LH-RH receptor,. . .

DETD (ii) contacting the recombinant human LH-RH receptor **protein**, a peptide fragment thereof or a salt thereof of the present invention with a labeled ligand to an LH-RH receptor. . .

DETD (i) contacting the CHO cells or membrane fractions thereof containing a recombinant human LH-RH receptor **protein** of the present invention with a labeled ligand to an LH-RH receptor, and

DETD (ii) contacting the CHO cells or membrane fractions thereof containing a recombinant human LH-RH receptor **protein** of the present invention with a labeled ligand to an LH-RH receptor and a test compound;

DETD . . . salt thereof, which comprises contacting the CHO cell or the cell membrane fraction thereof containing a recombinant human LH-RH receptor **protein** of the present invention with a test compound; and measuring Cell stimulation activities through the recombinant human LH-RH receptor (for. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth);

DETD (i) contacting the CHO cell or the cell membrane fraction thereof containing a recombinant human LH-RH receptor **protein** of the present invention with an LH-RH receptor agonist, and

DETD (ii) contacting the CHO cell or the cell membrane fraction thereof containing a recombinant human LH-RH receptor **protein** of the present invention with an LH-RH receptor agonist and a test compound,

DETD . . . generation of intracellular cAMP, generation of intracellular

cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth).

DETD In the above-mentioned screening method (1a) or (2a), a compound which binds to the recombinant human LH-RH receptor **protein** or a peptide fragment thereof or the CHO cell or a membrane fraction thereof of the present invention inhibits the binding of a ligand to an LH-RH receptor with the recombinant human LH-RH receptor **protein** can be selected as the compound or a salt thereof which has affinity for an LH-RH receptor.

DETD Further, in the above-mentioned screening method (2b), a compound which binds to the human LH-RH receptor **protein** to exhibit cell stimulation activities through the human LH-RH receptor (for example, activities enhancing or inhibiting arachidonic acid release, acetylcholine. . . generation of intracellular cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and cell growth) can be selected as a human LH-RH receptor agonistic compound. Of the LH-RH receptor agonistic compounds, compounds. . .

DETD . . . binding of an LH-RH receptor agonist to CHO cell or a membrane fraction thereof containing the recombinant human LH-RH receptor **protein** but does not have the cell stimulation activities can be selected as the human LH-RH receptor antagonistic compound.

DETD Prior to the acquisition of the CHO cells containing the recombinant human LH-RH receptor **proteins** of the present invention, there were no animal cells capable of highly expressing the recombinant human LH-RH receptor **proteins**. It was therefore impossible to efficiently screen compounds or a salt thereof which have affinity for the LH-RH receptor, especially. . . the CHO cells introduced by the human LH-RH receptor cDNA of the present invention can express the human LH-RH receptor **proteins** in large amounts, so that they are useful for the screening of the compounds which have affinity for the LH-RH. . . capable of proliferation in suspension of the present invention are suitable for large-scale cultivation of the recombinant human LH-RH receptor **proteins**.

DETD When the CHO cells expressing the human LH-RH receptor **proteins** are used in the screening methods of the present invention, the CHO cells can be fixed with glutaraldehyde, formalin, etc.. . .

DETD Examples of the test compounds include peptides, **proteins**, non-peptide compounds, synthetic compounds, fermented products, cell extracts, plant extracts and animal tissue extracts, which may be either novel compounds. . .

DETD Specifically, when the above-mentioned screening method (1a) or (2a) is conducted, the CHO cell containing the recombinant human LH-RH receptor **protein** or the cell membrane fraction thereof, or the recombinant human LH-RH receptor **protein** or the peptide fragment thereof according to the present invention is first suspended in a buffer solution suitable for screening. . .

DETD . . . when the above-mentioned screening method (2b) and (2c) are conducted, the cell stimulation activities through the recombinant human LH-RH receptor **protein** (for example, activities enhancing or inhibiting arachidonic acid release, acetylcholine release, fluctuation in intracellular Ca^{2+} concentration, generation of intracellular

cAMP, generation of intracellular cGMP, production of inositol phosphate, fluctuation in cell membrane potential, phosphorylation of intracellular **proteins**, activation of c-fos, a reduction in pH, chemotactic activity, activation of G **proteins** and secretion of hormones) and cell growth can be assayed by known methods or by use of commercial measuring kits. Specifically, the CHO cell containing the recombinant human LH-RH receptor **protein** is first cultivated on a multiwell plate. In conducting the screening, the medium is preliminarily replaced by a fresh medium. . . . a substance (for example, arachidonic acid) used as an indicator for the cell stimulation activities is difficult because of a **catabolic** enzyme contained in the cell, an inhibitor to the **catabolic** enzyme may be added to conduct the assay. Further, activity such as inhibition of cAMP production can be detected as. . . .

DETD The kit for screening of the present invention contains the CHO cell containing the recombinant human LH-RH receptor **protein** or the cell membrane fraction thereof, or the recombinant human LH-RH receptor **protein**, the peptide fragment thereof or a salt thereof according to the present invention.

DETD (I) Kits for screening containing the CHO cell containing the recombinant human LH-RH receptor **protein**

DETD (2) Recombinant Human LH-RH Receptor **Protein** Sample

DETD A sample obtained by cultivation of CHO cells (5.times.10.sup.4 cells/well) expressing a recombinant human LH-RH receptor **protein** in a 24-well plate at 37.degree. C. at 5% CO.sub.2 and 95% air for 2 days.

DETD (1) The CHO cells expressing the recombinant human LH-RH receptor **proteins** cultivated on the 24-well plate are washed once with 300 .mu.l of the assay buffer A, followed by addition of. . . .

DETD (2) Recombinant Human LH-RH Receptor **Protein** Sample

DETD A sample is membrane fraction of CHO cells expressing human LH-RH receptor **protein**. Samples can be prepared from the CHO cells described above and stored at -80.degree. C. prior to use.

DETD (1) The membrane fraction of CHO cells expressing human LH-RH receptor **protein** is diluted to an appropriate concentration (about 0.1 to 5000 .mu.g/ml, preferably about 1 to 500 .mu.g/ml), and dispensed each. . . .

DETD . . . kits for screening of the present invention are compounds inhibiting the binding of LH-RH to the recombinant human LH-RH receptor **proteins** of the present invention. The compounds are selected from test compounds such as peptides, **proteins**, non-peptide compounds, synthetic compounds, cell extracts, plant extracts and animal tissue extracts which may be novel or known. The compounds. . . .

DETD . . . receptor and therefore they are also useful as a recomposition to detect or assay a receptor expression cell, LH-RH receptor **protein** in a body.

DETD The CHO cells of the present invention wherein said cell is capable of continued production of the human LH-RH receptor **proteins** are cells capable of highly expressing the human LH-RH receptor **proteins**. In particular, the cells adapted to the CHO cells capable of proliferation in suspension are suitable for large-scale cultivation of the human LH-RH receptor **proteins**.

DETD . . . which have affinity for an LH-RH receptor using the CHO cells having ability to continue producing the human LH-RH receptor **proteins** or the cell membrane fractions thereof, or the recombinant human LH-RH receptor **proteins** or the peptide fragments thereof according to the present invention, the LH-RH receptor agonistic, superagonistic or antagonistic compounds can be. . . .

DETD [SEQ NO:1] Shows a nucleotide sequence of a cDNA coding for human LH-RH receptor **protein**.

DETD [SEQ NO: 2] Shows an amino acid sequence of human LH-RH receptor **protein**.

DETD . . . of a DNA oligomer for PCR which is used for a cloning of a cDNA coding for rat LH-RH receptor **protein**. This sequence is a partial nucleotide sequence of a cDNA coding for murine LH-RH receptor **protein**.

DETD . . . of a DNA oligomer for PCR which is used for a cloning of a cDNA coding for rat LH-RH receptor **protein**. This sequence is a partial nucleotide sequence of a cDNA coding for murine LH-RH receptor **protein**.

DETD . . . LH-RH receptor cDNA expression vector. This sequence contains a partial nucleotide sequence of a cDNA coding for human LH-RH receptor **protein**.

DETD . . . LH-RH receptor cDNA expression vector. This sequence contains a partial nucleotide sequence of a cDNA coding for human LH-RH receptor **protein**.

DETD . . . 2 hours, and then, concentrated to obtain a residue, which was distributed between ethyl acetate and an aqueous solution of **ammonium chloride**. The aqueous layer was extracted with ethyl acetate. The extracts were collected and washed with saline. After drying on MgSO.sub.4,. . .

DETD . . . the single cell by the limiting dilution method to obtain cell line CHO/L39 which stably expresses the human LH-RH receptor **protein**. This cell line was repeatedly cloned to obtain cell line CHO/L39-7 which expresses the receptor in higher amount. In this. . . makes it possible to obtain a cell line in which an introduced gene is amplified and which expresses the desired **protein** with higher amount.

DETD The LH-RH receptor **protein** activity of the CHO cells or the cell membrane fraction thereof was assayed by the following method:

DETD Cell line CHO/L39-7 (2.times.10.sup.7 cells) highly expressing the human LH-RH receptor **protein** obtained in Example 2 were cultivated in 100 ml of TE medium [a 1:1 (v/v) mixed medium of Daigo T. . .

DETD . . . ID NO:1:

ATGGCAAACAGTGCCTCTCCTGAACAGAATCAAAATCACTGTTCAGCCATCAACAACAGC60
ATCCCACTGATGCAGGGCAACCTCCCCACTCTGACCTTGTCTGGAAAGATCCGAGTGACG120
GTTACTTTCTTCCTTTTTCTGCTCTCTGCGACCTTTAATGCTTCTTTCTTGTTGAAACTT180
CAGAAGTGGACACAGAAGAAAGAGAAAGGGAAAAAGCTCTCAAGAATGAAGCTGCTCTTA240
AAACATCTGACCTTAGCCAACCTGTTGGAGACTCTGATTGTCATGCCACTGGATGGGATG300
TGGAACATTACAGTCCAATGGTATGCTGGAGAGTTACTCTGCAAAGTTCTCAGTTATCTA360
AAGCTTTTCTCCATGTATGCCCCAGCCTTCATGATGGTGGTGATCAGCCTGGACCGCTCC420
CTGGCTATCACGAGGCCCCTAGCTTTGAAAAGCAACAGCAAAGTCGGACAGTCCATGGTT480
GGCCTGGCCTGGATCCTCAGTAGTGTCTTTGCAGGACCACAGTTATACATCTTCAGGATG540
ATTCATCTAGCAGACAGCTCTGGACAGACAAAAGTTTTCTCTCAATGTGTAACACACTGC600
AGTTTTTCACAATGGTGGCATCAAGCATTTTATAACTTTTTCACCTTCAGCTGCCTCTTC660
ATCATCCCTCTTTTCATCATGCTGATCTGCAATGCAAAAATCATCTTCACCCTGACACGG720
GTCCTTCATCAGGACCCCCACGAACTACAACTGAATCAGTCCAAGAACAATATACCAAGA780
GCACGGCTGAAGACTCTAAAAATGACGGTTGCATTTGCCACTTCATTTACTGTCTGCTGG840
ACTCCCTACTATGTCCTAGGAATTTGGTATTGGTTTGATCCTGAAATGTTAAACAGGTTG900
TCAGACCCAGTAAATCACTTCTTCTTTCTCTTTGCCTTTTTAAACCCATGCTTTGATCCA960
CTTATCTATGGATATTTTTCTCTG984

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 328 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: **protein**
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
MetAlaAsnSerAlaSerProGluGlnAsnGlnAsnHisCysSerAla
151015
IleAsnAsnSerIleProLeuMetGlnGlyAsnLeuProThrLeuThr
202530
LeuSerGlyLysIleArgValThrValThrPhePheLeuPheLeuLeu
354045
SerAlaThrPheAsnAlaSerPheLeuLeuLysLeuGlnLysTrpThr
505560
GlnLysLysGluLysGlyLysLysLeuSerArgMetLysLeuLeuLeu
65707580
LysHisLeuThrLeuAlaAsnLeuLeuGluThrLeuIleValMetPro
859095
LeuAspGlyMetTrpAsnIleThrValGlnTrpTyrAlaGlyGluLeu
100105110
LeuCysLysValLeuSerTyrLeuLysLeuPheSerMetTyrAlaPro
115120125
AlaPheMetMetValValIleSerLeuAspArgSerLeuAlaIleThr
130135140
ArgProLeuAlaLeuLysSerAsnSerLysValGlyGlnSerMetVal
145150155160
GlyLeuAlaTrpIleLeuSerSerValPheAlaGlyProGlnLeuTyr
165170175
IlePheArgMetIleHisLeuAlaAspSerSerGlyGlnThrLysVal
180185190
PheSerGlnCysValThrHisCysSerPheSerGlnTrpTrpHisGln
195200205
AlaPheTyrAsnPhePheThrPheSerCysLeuPheIleIleProLeu
210215220
PheIleMetLeuIleCysAsnAlaLysIleIlePheThrLeuThrArg
225230235240
ValLeuHisGlnAspProHisGluLeuGlnLeuAsnGlnSerLysAsn
245250255
AsnIleProArgAlaArgLeuLysThrLeuLysMetThrValAlaPhe
260265270
AlaThrSerPheThrValCysTrpThrProTyrTyrValLeuGlyIle
275280285
TrpTyrTrpPheAspProGluMetLeuAsnArgLeuSerAspProVal
290295300
AsnHisPhePhePheLeuPheAlaPheLeuAsnProCysPheAspPro
305310315320
LeuIleTyrGlyTyrPheSerLeu
325
(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS:. . .

L15 ANSWER 70 OF 109 USPATFULL

AB There are disclosed certain novel compounds identified as benzo-fused lactams which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to increase the stature of those afflicted with a lack of

a normal secretion of natural growth hormone. Growth promoting compositions containing such benzo-fused lactams as the active ingredient therefore are also disclosed.

AN 97:88977 USPATFULL
TI Benzo-fused lactams promote release of growth hormone
IN Wyvratt, Matthew, Mountainside, NJ, United States
DeVita, Robert, Westfield, NJ, United States
Bochis, Richard, East Brunswick, NJ, United States
Schoen, William, Edison, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5672596 19970930 <--
WO 9405634 19940317 <--
AI US 1995-392961 19950418 (8)
WO 1993-US7791 19930818
19950418 PCT 371 date
19950418 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1992-936975, filed on 28 Aug 1992, now patented, Pat. No. US 5283241
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5835
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5672596 19970930 <--
WO 9405634 19940317 <--
SUMM 1. Increased rate of **protein** synthesis in all cells of the body;
SUMM . . . still further use of the disclosed novel benzo-fused lactam growth hormone secretagogues is in combination with IGF-1 to reverse the **catabolic** effects of nitrogen wasting as described by Kupfer, et al, J. Clin. Invest., 91, 391 (1993).
SUMM . . . These varied uses of growth hormone may be summarized as follows: stimulating growth hormone release in elderly humans; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system; treatment of retardation; acceleration of wound healing; accelerating. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS. Treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .
DETD . . . at room temperature overnight, then diluted with 1 L of methylene chloride and washed with 500 mL of saturated aqueous **ammonium chloride**, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over magnesium. . .
DETD . . . under nitrogen at -10.degree. C. The suspension was allowed to

warm slowly to room temperature over 12 hours then saturated **ammonium chloride** solution (1 L) was added followed by sufficient water (approximately 1 L) to dissolve the precipitate. The solution was extracted. . .

DETD . . . two hours then diluted with 350 mL of methylene chloride. The solution was washed with water (2.times.150 mL), saturated aqueous **ammonium chloride** (150 mL), saturated aqueous sodium bicarbonate (4.times.150 mL) and saturated aqueous sodium chloride (150 mL), dried over sodium sulfate and. . .

DETD . . . at 0.degree. C. for 15 minutes, the reaction mixture was diluted with 400 mL of ethyl acetate and 50% saturated **ammonium chloride**. The mixture was transferred to a separatory funnel and the aqueous layer was separated. The organic layer was washed with. . .

DETD . . . at room temperature for 30 minutes, diluted with 100 mL of ethyl acetate, washed with 25 mL of saturated aqueous **ammonium chloride**, 25 mL of saturated aqueous sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium sulfate,. . .

DETD . . . room temperature, the reaction mixture was diluted with 100 mL of ethyl acetate, washed with 25 mL of saturated aqueous **ammonium chloride**, 25 mL of saturated sodium bicarbonate and 25 mL of brine. The organic layer was removed, dried over magnesium sulfate,. . .

DETD . . . The reaction mixture was stirred for 1 hour then diluted with 150 mL of ethyl acetate, washed with saturated aqueous **ammonium chloride**, saturated aqueous sodium bicarbonate, saturated aqueous sodium chloride, dried over magnesium sulfate and filtered. The solvent was removed under vacuum. . .

DETD . . . ether (4.14 mol, 1.5 eq.). The suspension was allowed to warm slowly to room temperature over 12 hours then saturated **ammonium chloride** solution (1 L) was added followed by sufficient water (approximately 1 L) to dissolve the precipitate. The solution was extracted. . .

DETD . . . C. for 3 hours then cooled to room temperature. The reaction mixture was diluted with 100 mL of saturated aqueous **ammonium chloride**, transferred to a separatory funnel and extracted with ether (3.times.150 mL). The combined ether extracts were washed with saturated aqueous. . .

DETD . . . 310 mg (0.73 mmol) 2-benzyloxycarbonylamino-2-methyl-N-[7-nitro-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepin-3(R)-yl]propanamide (Step B) in 20 mL of methanol was added 78 mg (1.5 mmol) of **ammonium chloride** followed by 669 mg (10.2 mmol) of zinc dust. The resulting mixture was heated at reflux for four hours. The. . .

CLM What is claimed is:
21. A method for the treatment of the **catabolic** effects of nitrogen wasting which comprises administering to such patient a compound of claim 1 in combination with insulin-like growth. . .
22. A composition for the treatment of the **catabolic** effects of nitrogen wasting which comprises an inert carrier and a compound of claim 1 in combination with insulin-like growth. . .

L15 ANSWER 71 OF 109 USPATFULL

AB The present invention is directed to certain compounds of the general structural formula: ##STR1## wherein R.sub.1, R.sub.1a, R.sub.2a, R.sub.3, R.sub.3a, R.sub.4, R.sub.5, R.sub.6, A, W, and n are as defined herein. These compounds promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food

animals to render the production of edible meat products more
efficient,
and in humans, to treat physiological or medical conditions
characterized by a deficiency in growth hormone secretion, such as
short
stature in growth hormone deficient children, and to treat medical
conditions which are improved by the anabolic effects of growth
hormone.
Growth hormone releasing compositions containing such compounds as the
active ingredient thereof are also disclosed.

AN   97:78440 USPATFULL
TI   Acyclic compounds promote release of growth hormone
IN   Chen, Meng Hsin, Westfield, NJ, United States
        Morriello, Gregori J., Belleville, NJ, United States
        Nargund, Ravi, East Brunswick, NJ, United States
        Patchett, Arthur A., Westfield, NJ, United States
        Yang, Lihu, Edison, NJ, United States
PA   Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI   US 5663171   19970902   <--
AI   US 1995-398247   19950303 (8)
RLI   Continuation-in-part of Ser. No. US 1993-157774, filed on 24 Nov 1993,
        now abandoned
DT   Utility
FS   Granted
EXNAM   Primary Examiner: Springer, David B.
LREP   Thies, J. Eric, Rose, David L.
CLMN   Number of Claims: 9
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 2352
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI   US 5663171   19970902   <--
SUMM   . . . hormone is known to have the following basic effects on the
        metabolic processes of the body: (1) Increased rate of **protein**
        synthesis in all cells of the body; (2) Decreased rate of carbohydrate
        utilization in cells of the body; (3) Increased. . .
SUMM   . . . e.g., an antibiotic growth permittant or an agent to treat
        osteoporosis or in combination with a corticosteroid to minimize the
        catabolic side effects or with other pharmaceutically active
        materials wherein the combination enhances efficacy and minimizes side
        effects.
SUMM   . . . uses as growth hormone itself. These varied uses may be
        summarized as follows: treating growth hormone deficient adults;
        prevention of **catabolic** side effects of glucocorticoids;
        treatment of osteoporosis; stimulation of the immune system,
        acceleration of wound healing; accelerating bone fracture repair;. .
.
        syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound
        healing, and psychosocial deprivation; treatment of pulmonary
        dysfunction and ventilator dependency; attenuation of **protein**
        catabolic response after a major operation; treating
        malabsorption syndromes; reducing cachexia and **protein** loss
        due to chronic illness such as cancer or AIDS; accelerating weight gain
        and **protein** accretion in patients on TPN (total parenteral
        nutrition); treatment of hyperinsulinemia including nesidioblastosis;
        adjuvant treatment for ovulation induction and to. . . virus;
        treatment of syndromes manifested by non-restorative sleep and
        musculoskeletal pain, including fibromyalgia syndrome or chronic
fatigue
        syndrome; improvement in **muscle** strength, mobility,

maintenance of skin thickness, metabolic homeostasis, renal hemeostasis
in the frail elderly; stimulation of osteoblasts, bone remodelling,
and.

. .

SUMM . . . the instant compounds are useful in the prevention or
treatment
of a condition selected from the group consisting of: osteoporosis;
catabolic illness; immune deficiency, including that in
individuals with a depressed T.sub.4 /T.sub.8 cell ratio; hip fracture;
musculoskeletal impairment in the elderly; growth hormone deficiency in
adults or in children; obesity; cachexia and **protein** loss due
to chronic illness such as AIDS or cancer; and treating patients
recovering from major surgery, wounds or burns,. . .

DETD . . . TiCl.sub.4 (0.46 ml) was added. After stirring 2.5 hour at
0.degree. C., this clear solution was quenched with saturated aqueous
ammonium chloride. This mixture was extracted with
methylene chloride, washed with sodium bicarbonate, brine and dried
over
sodium sulfate. Concentration and purification. . .

L15 ANSWER 72 OF 109 USPATFULL

AB There are disclosed certain novel compounds identified as spiro
piperidines and homologs which promote the release of growth hormone in
humans and animals. This property can be utilized to promote the growth
of food animals to render the production of edible meat products more
efficient, and in humans, to treat physiological or medical conditions
characterized by a deficiency in growth hormone secretion, such as
short
stature in growth hormone deficient children, and to treat medical
conditions which are improved by the anabolic effects of growth
hormone.
Growth hormone releasing compositions containing such spiro compounds
as
the active ingredient thereof are also disclosed.

AN 97:66122 USPATFULL

TI Method of using spiro piperidines to promote the release of growth
hormone

IN Chen, Meng-Hsin, Westfield, NJ, United States
Nargund, Ravi P., East Brunswick, NJ, United States
Johnston, David B. R., Warren, NJ, United States
Patchett, Arthur A., Westfield, NJ, United States
Tata, James R., Westfield, NJ, United States
Yang, Lihu, Edison, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5652235 19970729 <--

AI US 1996-641311 19960430 (8)

RLI Division of Ser. No. US 1993-147226, filed on 3 Nov 1993, now patented,
Pat. No. US 5536716 which is a continuation-in-part of Ser. No. US
1992-989322, filed on 11 Dec 1992, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Covington,
Raymond

LREP Thies, J. Eric, Rose, David L.

CLMN Number of Claims: 9

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 4024

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5652235 19970729 <--

SUMM 1. Increased rate of **protein** synthesis in all cells of the body;

SUMM . . . e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the **catabolic** side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.

SUMM . . . hormone may be summarized as follows: stimulating growth hormone release in elderly humans; treating growth hormone deficient adults; prevention of **catabolic** side effects of glucocorticoids, treatment of osteoporosis, stimulation of the immune system, acceleration of wound healing, accelerating bone fracture repair,. . . syndrome, sleep disorders, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; treating malabsorption syndromes, reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS; accelerating weight gain and **protein** accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to. . . adjunctive therapy for patients on chronic hemodialysis; treatment of immunosuppressed patients and to enhance antibody response following vaccination; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .

DETD . . . (1.85 mmol) was added and stirred for 3 h. The reaction mixture was poured into 15 mL of saturated aqueous **ammonium chloride** solution and extracted with ether (2.times.15 mL). The combined organics were washed with water (15 mL), brine (15 mL), dried. . .

L15 ANSWER 73 OF 109 USPATFULL

AB There are disclosed certain novel compounds identified as heterocyclic-fused lactams which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to increase the stature of those afflicted with a lack of a normal secretion of natural growth hormone. Growth promoting compositions containing such heterocyclic-fused lactams as the active ingredient thereof are also disclosed.

AN 97:16205 USPATFULL
TI Heterocyclic-fused lactams promote release of growth hormone
IN Fisher, Michael H., Ringoes, NJ, United States
Mrozik, Helmut, Matawan, NJ, United States
Schoen, William R., Edison, NJ, United States
Shih, Thomas L., Edison, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5606054 19970225 <--
AI US 1993-166440 19931214 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Thies, J. Eric, Rose, David L.
CLMN Number of Claims: 6

ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1768
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5606054 19970225 <--
SUMM 1. Increased rate of **protein** synthesis in all cells of the body;
SUMM . . . still further use of the disclosed novel heterocyclic-fused lactam growth hormone secretagogues is in combination with IGF-1 to reverse the **catabolic** effects of nitrogen wasting as described by Kupfer, et al, J. Clin. Invest., 91, 391 (1993).
SUMM . . . These varied uses of growth hormone may be summarized as follows: stimulating growth hormone release in elderly humans; prevention of **catabolic** side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune s system; treatment of retardation; acceleration of wound healing;. . . syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of **protein catabolic** response after a major operation; reducing cachexia and **protein** loss due to chronic illness such as cancer or AIDS. Treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function; treatment of immunosuppressed patients; improvement in **muscle** strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and. . .
DETD . . . at room temperature overnight, then diluted with 1 L of methylene chloride and washed with 500 mL of saturated aqueous **ammonium chloride**, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over magnesium. . .
DETD . . . dispersion. After 2 min, 60 mg of N-triphenylmethyl-5-[2-(4'-bromomethylbiphen-4-yl)] tetrazole was added. After an additional 5 min, ice and saturated aqueous **ammonium chloride** solution was added to stop the reaction. The products were extracted with ethyl acetate and purified by PTLC on silica. . .

L15 ANSWER 74 OF 109 USPATFULL
AB A formulation for IGF-I is disclosed that is useful in treating hyperglycemic disorders and, in combination with growth hormone, in enhancing growth of a mammal. Also disclosed is a process for preparing a formulation of growth hormone and IGF-I from the IGF-I formulation. The IGF-I formulation comprises about 2-20 mg/ml of IGF-I, about 2-50 mg/ml of an osmolyte, about 1-15 mg/ml of a stabilizer, and a buffered solution at about pH 5-5.5, optionally with a surfactant.
AN 97:7906 USPATFULL
TI Method of formulating IGF-I with growth hormone
IN Clark, Ross G., Pacifica, CA, United States
Yeung, Douglas A., Fremont, CA, United States
Oeswein, James Q., Moss Beach, CA, United States
PA Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 5597802 19970128 <--
AI US 1995-458595 19950602 (8)
RLI Division of Ser. No. US 1993-71819, filed on 4 Jun 1993 which is a

continuation-in-part of Ser. No. US 1991-806748, filed on 13 Dec 1991,
now abandoned which is a division of Ser. No. US 1990-535005, filed on
7
Jun 1990, now patented, Pat. No. US 5126324
DT Utility
FS Granted
EXNAM Primary Examiner: Schain, Howard E.
LREP Hasak, Janet E.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN 28 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 2296
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5597802 19970128 <--
SUMM . . . for example, blood and human cerebral spinal fluid. Most tissues and especially the liver produce IGF-I together with specific IGF-binding **proteins**. These molecules are under the control of growth hormone (GH). Like GH, IGF-I is a potent anabolic **protein** . See Tanner et al., Acta Endocrinol., 84: 681-696 (1977); Uthne et al., J. Clin. Endocrinol. Metab., 39: 548-554 (1974)). IGF-I. . .
SUMM . . . J. Lab. Clin. Med., 49: 825-836 (1957). Many studies investigating the relationships among GH, IGF-I, cartilage, cultured human fibroblasts, skeletal **muscle**, and growth have supported this somatomedin hypothesis. See, e.g., Phillips and Vassilopoulou-Sellin, N. Engl. J. Med., 302: 372-380; 438-446 (1980);. . .
SUMM Various methods for formulating **proteins** or polypeptides have been described. These include EP 267,015 published May 11, 1988; EP 308,238 published Mar. 22, 1989; and. . . 193,917 published Sep. 10, 1986, which discloses a slow-release composition of a carbohydrate polymer such as a cellulose and a **protein** such as a growth factor; GB Pat. No. 2,160,528 granted Mar. 9, 1988, describing a formulation of a bioactive **protein** and a polysaccharide; and EP 193,372 published Sep. 3, 1986, disclosing an intranasally applicable powdery pharmaceutical composition containing an active. . . synthetic polymers able to chelate Ca and Mg; and JP 57/026625 published Feb. 12, 1982 disclosing a preparation of a **protein** and water-soluble polymer such as soluble cellulose.
DETD . . . the exception of the presence of an N-terminal methionine residue. This added amino acid is a result of the bacterial **protein** synthesis process.
DETD . . . has been blocked chemically (i.e., by glucocorticoid treatment) or by a natural condition such as in adult patients or in **catabolic** patients where the IGF-I response to GH is naturally reduced.
DETD In addition, the IGF-I is suitably administered together with its binding **protein**, for example, BP53, which is described in WO 89/09268 published Oct. 5, 1989, which is equivalent to U.S. Ser. No.. . . which are incorporated herein by reference. This administration may be by the method described in U.S. Pat. No. 5,187,151. This **protein** is an acid-stable component of about 53 Kd on a non-reducing SDS-PAGE gel of a 125-150 Kd glycoprotein complex found. . . GH can be delivered coupled to another agent such as an antibody, an antibody fragment, or one of its binding **proteins**.
DETD . . . their salts; antioxidants such as ascorbic acid; low molecular

weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeprides; **proteins**, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic. . .

DETD . . . These salts are useful as surface-active germicides for many pathogenic non-sporulating bacteria and fungi and as stabilizers. Examples include octadecyldimethylbenzyl **ammonium chloride**, hexamethonium-chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other. . .

DETD The "inorganic salt" is a salt that does not have a hydrocarbon-based cation or anion. Examples include sodium chloride, **ammonium chloride**, potassium chloride, magnesium chloride, calcium chloride, sodium phosphate, calcium phosphate, magnesium phosphate, potassium phosphate, ammonium phosphate, sodium sulfate, ammonium sulfate,. . .

DETD . . . the final pH will not vary significantly from 5.4 in the final IGF-I/GH mixture to maintain good solubility of both **proteins** over a wide mixing ratio range. However, a broader pH range in terms of stability of both **proteins** is from about 5 to about 6.

DETD . . . associated with aging such as increasing lean mass to fat ratios, immuno-deficiencies including increasing CD4 counts and increasing immune tolerance, **catabolic** states associated with wasting, etc., Laron dwarfism, insulin resistance, and so forth.

DETD . . . mixed with hGH in dose ratios of IGF-I:hGH of greater than about 2:1 to provide a stable co-mix of both **proteins**. In this example, the IGF-I formulation used to achieve this was:

DETD . . . from the blood. The IGF-I concentration in the plasma samples was measured (after acid-ethanol extraction to remove the IGF binding **proteins**) by radioimmunoassay.

DETD . . . as much (230-250 grams) as the dw/dw rats, and might be expected to have higher concentrations of plasma IGF binding **proteins**, the doses of IGF-I were doubled, compared to those used in the earlier examples in the dw/dw rat.

L15 ANSWER 75 OF 109 MEDLINE

AB A Chinese hamster ovary (CHO) cell line producing a recombinant glycoprotein was cultured in batch mode with different initial concentrations of **ammonium chloride** (0-10 mM), sodium lactate (0-60 mM), or sodium chloride (0-60 mM). High ammonium concentrations did not inhibit cell growth and productivity or glucose and glutamine consumption. In contrast, specific ammonia and alanine production decreased by 55% and 40%, respectively. There were also significant increases in specific aspartate and glutamate consumption in high ammonium concentrations. These observations indicated a shift in glutamine **catabolic** pathways in response to the effects of ammonium. The influence of lactate on growth and metabolism were the combined effects of lactate concentration and osmolarity. After "correcting" for osmolarity effects, lactate was found to inhibit growth by 25% but to increase specific productivity slightly (10%). Lactate had profound effects not only on glycolysis but also on glutaminolysis. While specific glucose and glutamine consumptions decreased by 15-20%, the effects of lactate on their metabolic products were far more significant. Lactate production was halted, and specific ammonia and alanine productions decreased by 64% and 70% at high lactate concentration. Theories on how ammonium and lactate affected the metabolic pathways of glucose and glutamine are presented.

AN 97478076 MEDLINE

DN 97478076 PubMed ID: 9336989
TI Effects of ammonium and lactate on growth and metabolism of a recombinant Chinese hamster ovary cell culture.
AU Lao M S; Toth D
CS Cangene Corporation, Winnipeg, Manitoba, Canada.

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L5   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2002 ACS
RN   34747-66-5  REGISTRY
CN   L-Glutamic acid, N,N'-1,2-ethanediylbis- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Ethylenediamine-N,N'-diglutaric acid
CN   L,L-N,N'-Ethylenediglutamic acid
CN   N,N'-Ethylenediaminebis(.alpha.-glutaric acid)
FS   STEREOSEARCH
DR   51006-56-5, 36011-42-4
MF   C12 H20 N2 O8
CI   COM
LC   STN Files:   BEILSTEIN*, CA, CAPLUS, CASREACT, TOXCENTER, USPAT2,
       USPATFULL
         (*File contains numerically searchable property data)
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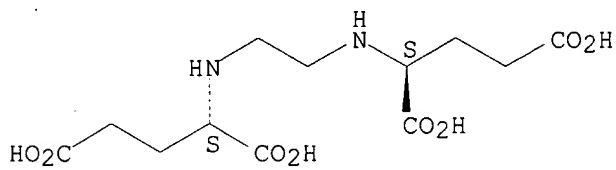
Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

26 REFERENCES IN FILE CA (1962 TO DATE)
4 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
26 REFERENCES IN FILE CAPLUS (1962 TO DATE)

Vinnars

$$\overset{144}{(12 \times 12)} + \overset{20}{(1 \times 20)} + \overset{28}{(2 \times 14)} + \overset{128}{(16 \times 8)} = 320\ g/\phi$$

$$\frac{0.1g / 1}{320g / 1\phi} = \boxed{3.125 \times 10^{-4}\ \phi \text{ of AKG}} = 3.125 \times 10^{-4} \times 10^{6} = 3.125 \times 10^{2} = 312.5\ \mu\phi/\text{Kg body wt./day}$$

3.125×10^{-4} g/Kg body wt./day